ALTERNATING CURRENT POLAROGRAPHY OF ORGANIC COMPOUNDS

VI. FURTHER DEVELOPMENT OF THE THEORY

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Summary

In previous papers of this series (Breyer, Bauer, and Hacobian 1954; Breyer and Bauer 1956) it was shown that the A.C. polarographic behaviour of organic compounds can be explained by postulating adsorption of the substances at the electrode. Empirical and theoretical expressions for the dependence of the alternating current on the bulk concentration of depolarizer were qualitatively correlated. In the present paper, the theoretical treatment is developed further, permitting the evaluation of the adsorption coefficients of the oxidized and reduced forms at the electrode surface.

I. INTRODUCTION

In Part V of this series (Breyer and Bauer 1956), the results obtained in A.C. polarography of organic compounds were discussed on the basis of adsorption processes. An equation was derived to express the fraction of the surface of the electrode covered by adsorbed molecules. It was assumed that the amounts of oxidized and reduced forms in the adsorbed state were equal at the summit potential (E_s). The relevant equation (eqn. (7), Part V) is

$$\theta_{(E_s)}=\frac{b_{\mathrm{Ox}}xC}{1+b''_{\mathrm{R}}C+(2b_{\mathrm{Ox}}-b'_{\mathrm{Ox}}-b''_{\mathrm{R}})xC}=\frac{b_{\mathrm{R}}(1-x)C}{1+(2b_{\mathrm{R}}-b'_{\mathrm{R}})C+(b''_{\mathrm{Ox}}+b'_{\mathrm{R}}-2b_{\mathrm{R}})xC}, \quad \ldots\ldots\ldots\ldots (1)$$

where $\theta_{(E_s)}$ is the fraction of the surface covered by oxidized (or reduced) adsorbed molecules at E_s; C is the total bulk concentration of depolarizer; b_{Ox}, b'_{Ox}, b''_{Ox}, b_{R}, b'_{R}, b''_{R} are adsorption coefficients; x is defined by

$$C_{\mathrm{Ox\ II}(E_s)}=xC, \quad C_{\mathrm{R\ II}(E_s)}=(1-x)C, \quad \ldots\ldots\ldots (2)$$

where $C_{\mathrm{Ox\ II}(E_s)}$, $C_{\mathrm{R\ II}(E_s)}$ are the concentrations of oxidized, reduced forms respectively, unadsorbed, at the electrode at E_s.

It was not possible to obtain an expression for x in a usable form from equation (1). However, for the special case where x is independent of C, it was shown that

$$x=\frac{b_{\mathrm{R}}}{b_{\mathrm{Ox}}+b_{\mathrm{R}}}, \quad \ldots\ldots\ldots\ldots\ldots (3)$$

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and

$$\frac{b_{Ox}}{b_R}=\frac{b'_{Ox}}{b'_R}=\frac{b''_{Ox}}{b''_R}. \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

This latter identity is obeyed when the adsorption properties of oxidized and reduced forms are the same. In this case equation (1) reduces to

$$\theta_{(E_s)}=\frac{1}{2}\cdot\frac{bC}{1+bC}, \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

where $b_{Ox}=b_R=b$.

It was also shown that

$$\frac{\theta_{Ox}}{\theta_R}=\frac{C_{Ox\,I}}{C_{R\,I}}=\frac{C_{Ox\,II}}{C_{R\,II}}\cdot\frac{b_{Ox}+b_{Ox}b'_{Ox}C_{Ox\,II}+b'_{Ox}b_RC_{R\,II}}{b_R+b_{Ox}b'_RC_{Ox\,II}+b_Rb'_RC_{R\,II}}, \quad \ldots. (6)$$

(eqns. (4) and (17) of Part V), so that, in general, $C_{Ox\,I}/C_{R\,I}\neq C_{Ox\,II}/C_{R\,II}$. For the special case where x is independent of C and $b_{Ox}=b_R$, equation (6) reduces to

$$\frac{C_{Ox\,I}}{C_{R\,I}}=\frac{C_{Ox\,II}}{C_{R\,II}}. \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Equally, the theoretical expression for the height of the A.C. polarographic wave of an organic substance was given in Part V as

$$i_{\sim(E_s)}=\frac{yb_{Ox}xC}{1+b''_RC+(2b_{Ox}-b'_{Ox}-b''_R)xC}=\frac{yb_R(1-x)C}{1+(2b_R-b'_R)C+(b''_{Ox}+b'_R-2b'_R)xC}, \quad \ldots\ldots\ldots\ldots\ldots\ldots (8)$$

where y is a proportionality constant. For the case where x is independent of C and $b_{Ox}=b_R=b$, this reduces to

$$i_{\sim(E_s)}=\frac{1}{2}\cdot\frac{ybC}{1+bC}. \quad \ldots\ldots\ldots\ldots\ldots\ldots (9)$$

The experimentally-observed relation between A.C. wave height and bulk concentration is

$$i_{\sim(E_s)}=\frac{abC}{1+bC}\left(1+\frac{\alpha\beta C}{1+\beta C}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots (10)$$

(eqn. (22) in Part V), where a, b, α, β are empirical parameters and α may be positive, negative, or zero. In the latter case the empirical equation is

$$i_{\sim(E_s)}=\frac{abC}{1+bC} \quad \ldots\ldots\ldots\ldots\ldots\ldots (11)$$

(eqn. (23) in Part V).

Theory and experiment were compared by correlating equations (8) and (10), and equations (9) and (11).

II. Further Development of the Theory

For the case where x is independent of C, but without assuming that $b_{Ox}=b_R=b$, it is possible to obtain expressions more general than equations (5), (7), and (9) by substituting equations (3) and (4) directly into equations (1), (6), and (8). These general solutions for the case where x is independent of C are

$$\theta_{(E_s)}=\frac{b_{Ox}b_R}{b_{Ox}+b_R}C\Big/\left(1+2\frac{b_{Ox}b_R}{b_{Ox}+b_R}C\right), \quad \ldots\ldots\ldots (12)$$

$$\frac{C_{Ox\,I}}{C_{R\,I}}=\frac{b_{Ox}C_{Ox\,II}}{b_RC_{R\,II}}, \quad \ldots\ldots\ldots\ldots\ldots\ldots (13)$$

and

$$i_{\sim(E_s)}=y\frac{b_{Ox}b_R}{b_{Ox}+b_R}C\Big/\left(1+2\frac{b_{Ox}b_R}{b_{Ox}+b_R}C\right). \quad \ldots\ldots\ldots (14)$$

The correlation of equation (8) with equation (10) can be tested specifically by investigating what values of x and of the adsorption coefficients will satisfy the empirical equation (10). This was done as follows:

(i) x was eliminated by combining equation (10) with the two forms of equation (8). The resulting expression contains terms of C to the powers of 0, 1, 2, 3, 4.

(ii) Since the relation is valid for all values of C, coefficients of C^0, C^1, etc. can be equated. In the resulting five equations, a, b, α, β, and y were progressively eliminated.

When the final quadratic equation was solved, two possible solutions were obtained (1):

$$b'_{Ox}=-b''_{Ox}, \text{ and } b'_R=-b''_R,$$

and (2):

$$\frac{b_{Ox}}{b_R}=\frac{b'_{Ox}}{b''_R}=\frac{b''_{Ox}}{b'_R}.$$

The first solution has no physical significance, since b'_{Ox}, b''_{Ox}, b'_R, and b''_R are all positive quantities. The second solution is the same as that obtained in the previous treatment for x independent of C (eqn. (4)), and, therefore, leads to equation (14) as the expression for the height of the A.C. wave. Thus equation (14), which is of the same form as the empirical equation (11), arises as a necessary conclusion from the correlation of equations (8) and (10) and not, as in the previous treatment, as an arbitrary special case.

In the light of this result the experimental curves were re-examined. It was found that three of the curves previously fitted to equation (10) may equally be fitted to the simpler expression (11) (see Table 1).

However, the more negative wave of chloranilic acid at pH 2·2 cannot be fitted to equation (11). It is noteworthy that in this case both the A.C. and D.C. polarograms show two waves, which have been ascribed to the reduction of two different ionic species of chloranilic acid (Breyer and Bauer 1955). Whilst the more positive wave yields a curve which follows equation (11), the more

TABLE 1

A.C. WAVE HEIGHT AND BULK CONCENTRATION CHLORANILIC ACID IN "UNIVERSAL" BUFFERS*

Concentration (M)	A.C. Wave Height (μA)		
	Observed	Calc. from Eqn. (10)	Calc. from Eqn. (11)
pH 4·9 at 20 °C			
$3{\cdot}25\times10^{-7}$	0·73	$0{\cdot}53_5$	0·55
6·25	1·06	0·98	1·01
$1{\cdot}15\times10^{-6}$	1·6	1·7	1·75
2·05	2·5	2·9	2·85
3·1	4·5	4·1	3·9
8·35	8·75	7·6	7·05
$1{\cdot}65\times10^{-5}$	10·85	9·9	9·3
3·25	10·7	11·0	11·05
6·25	10·3	11·15	12·15
$1{\cdot}15\times10^{-4}$ ..	10·1	10·8	12·85
2·05	10·3	10·2	13·2
3·8	11·2	$9{\cdot}7_5$	13·4
8·4	14·0	$9{\cdot}7_5$	13·6
Average deviation from experiment		12%	15%
			$a=13{\cdot}4$ μA
			$b=1{\cdot}28\times10^5$ l. mole^{-1}
pH 4·9 at 30° C			
$6{\cdot}75\times10^{-7}$	0·7	0·61	0·68
$1{\cdot}4\times10^{-6}$	1·15	1·15	1·26
2·7	1·75	2·0	2·1
5·1	3·2	3·25	3·2
7·4	3·8	4·05	3·9
8·9	4·9	4·5	4·25
$1{\cdot}45\times10^{-5}$	5·9	5·55	5·1
1·6	5·7	5·7	5·25
2·65	6·5	6·5	5·95
2·85	7·05	6·6	6·0
5·5	7·0	7·0	6·65
9·7	6·8	7·0	6·95
$1{\cdot}7\times10^{-4}$	6·6	6·85	7·15
2·85	6·6	6·75	7·3
3·7	6·6	6·75	7·3
4·95	6·9	6·65	7·35
7·45	7·2	6·6	7·4
Average deviation from experiment		4·6%	8·4%
			$a=7{\cdot}45$ μA
			$b=1{\cdot}49\times10^5$ l. mole^{-1}

* Prideaux and Ward (1924).

TABLE 1 (*Continued*)

Concentration (M)	A.C. Wave Height (μA)		
	Observed	Calc. from Eqn. (**10**)	Calc. from Eqn. (**11**)
pH 7·1 at 20 °C			
$5{\cdot}5\times10^{-7}$	0·18	0·15	0·15
$1{\cdot}05\times10^{-6}$	0·28	0·29	0·29
2·0	0·53	0·53	0·53
3·5	0·9	0·9	0·40
$1{\cdot}45\times10^{-5}$	2·83	3·01	2·99
2·8	4·75	4·7	4·65
5·5	6·5	6·5	6·55
$1{\cdot}05\times10^{-4}$ ..	8·2	8·05	8·2
2·0	4·0	9·15	9·45
3·5	10·0	9·8	10·2
6·2	10·4	10·2	10·7
$1{\cdot}45\times10^{-3}$ ..	10·9	10·5	11·1
Average deviation from experiment		3·6%	3·6%
		$a=11{\cdot}4$ μA	
		$b=2{\cdot}45\times10^{4}$ l. mole $^{-1}$	

negative one follows equation (**10**), α having a negative value. This can be explained by assuming that the reduced form produced during the first reduction step remains adsorbed at those more negative potentials, where the second species is reduced. The form of equation (**10**) can then be simply explained by regarding the term $1-\alpha\beta C/(1+\beta C)$ as the fraction of the surface free for the electrochemical reduction of the second ionic species. The term $\alpha\beta C/(1+\beta C)$ would then represent the fraction of the surface covered by the reduced form of the first species.

The present treatment permits evaluation of the Langmuir adsorption coefficients of the oxidized and reduced forms of the depolarizer. Since

$$C_{\text{Ox II }(E_g)}=K(i_d-i_{(E_g)}),$$

and

$$C_{\text{R II }(E_g)}=Ki_{(E_g)},$$

for a diffusion-controlled process, and using equations (**2**) and (**3**), it follows that

$$\frac{b_{\text{Ox}}}{b_{\text{R}}}=\frac{i_{(E_g)}/i_d}{1-i_{(E_g)}/i_d}. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (\mathbf{15})$$

Also, correlating equations (**11**) and (**14**), the empirical parameter b is given by

$$b=\frac{2b_{\text{Ox}}b_{\text{R}}}{b_{\text{Ox}}+b_{\text{R}}}. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (\mathbf{16})$$

Thus the values of b_{Ox} and b_R, the Langmuir adsorption coefficients of oxidized and reduced forms of the depolarizer, can be determined for an organic substance by measurement of the quantities b and $i_{(E_g)}/i_d$, as shown in Table 2.

TABLE 2

VALUES OF THE ADSORPTION COEFFICIENTS " UNIVERSAL " BUFFERS AT 20 °C

Substance	i_{E_g}/i_d (empirical)	b (empirical) (l. mole^{-1})	b_{Ox} (evaluated) (l. mole^{-1})	b_R (evaluated) (l. mole^{-1})
Quinone				
pH 7·3 ..	0·33	$5{\cdot}47\times10^{3}$	$4{\cdot}05\times10^{3}$	$8{\cdot}3\times10^{3}$
pH 9·0 ..	0·22	$3{\cdot}70\times10^{3}$	$2{\cdot}35\times10^{3}$	$8{\cdot}45\times10^{3}$
Chloranilic acid				
pH 4·9 ..	0·68	$1{\cdot}28\times10^{5}$	$2{\cdot}0\times10^{5}$	$9{\cdot}45\times10^{4}$
pH 7·1 ..	0·65	$2{\cdot}45\times10^{4}$	$3{\cdot}5\times10^{4}$	$1{\cdot}9\times10^{4}$
*peri*Naphthenone				
pH 2·9 ..	0·43	$1{\cdot}50\times10^{4}$	$1{\cdot}3\times10^{4}$	$1{\cdot}75\times10^{4}$
pH 3·9 ..	0·43	$5{\cdot}65\times10^{3}$	$4{\cdot}95\times10^{3}$	$6{\cdot}6\times10^{3}$
pH 5·1 ..	0·35	$3{\cdot}20\times10^{3}$	$2{\cdot}45\times10^{3}$	$4{\cdot}55\times10^{3}$
pH 6·0 ..	0·415	$6{\cdot}15\times10^{3}$	$5{\cdot}25\times10^{3}$	$7{\cdot}4\times10^{3}$
pH 7·0 ..	0·315	$5{\cdot}25\times10^{3}$	$3{\cdot}8\times10^{3}$	$8{\cdot}35\times10^{3}$

III. REFERENCES

BREYER, B., and BAUER, H. H. (1955).—*Aust. J. Chem.* **8**: 472.
BREYER, B., and BAUER, H. H. (1956).—*Aust. J. Chem.* **9**: 1.
BREYER, B., BAUER, H. H., and HACOBIAN, S. (1954).—*Aust. J. Chem.* **7**: 305.
PRIDEAUX, E. B. R., and WARD, A. T. (1924).—*J. Chem. Soc.* **125**: 426.

DIRECT CURRENT MEASUREMENTS OF THE CONDUCTANCE OF SOLUTIONS OF SODIUM AND POTASSIUM CHLORIDE AT 25 °C

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Summary

Measurements of the conductance of concentrated solutions of sodium chloride and potassium chloride at 25 °C have been made by the D.C. method. They have been interpreted in the light of recent theories with special reference to the effect of viscosity.

I. Introduction

On account of its comparative simplicity, the D.C. method of measuring electrolytic conductance (Gunning and Gordon 1942) is receiving consideration as a possible substitute for the A.C. method ; even if it is not superior it should be at least a useful complement to the more conventional method. Jones and Josephs (1928) and Jones and Bollinger (1929) have shown that the A.C. method requires elaborate precautionary measures against extraneous capacitative and inductive effects, the need to ensure a sinusoidal waveform, and the choice of a suitable thermostat liquid ; moreover, the nature of the frequency effect, although admittedly of a second order, is not well understood. The primary purpose of the alternating current is to counteract polarization of the electrodes, although, strictly speaking, this purpose is achieved only at infinite frequency ; in view of the apparent increase in resistance (reactance) with increasing frequency, extrapolation to infinite frequency involves some measure of uncertainty. The D.C. method overcomes this difficulty by using reversible electrodes, and the contribution of Ives and Swaroopa (1953) consisted of placing the probe-electrodes in regions of zero potential gradient, thereby obviating the need to locate the electrodes accurately and reproducibly, as in Gordon's method.

The concordance between data such as those on sodium chloride by Shedlovsky (1932), using the A.C. bridge method, and those by Gunning and Gordon (1942), using the D.C. method, shows that the two methods lead to the same result *in dilute solution*, but it is not known if this concordance extends to concentrated solutions and it may be noted that Eastman (1920) observed a difference of 0·03 per cent. between the two methods. How this difference depends on frequency has not been studied and Blake's (1950) work in the radio-frequency range appears to offer possibilities.

Wishaw and Stokes (1954) have recently shown that the introduction of a simple viscosity factor into Falkenhagen, Leist, and Kelbg's (1952) extension of the Debye-Hückel-Onsager conductivity equation gives remarkable con-

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cordance with experiment up to unexpectedly high concentrations. To study the relation between viscosity and conductance in concentrated solutions, we have now made measurements on sodium and potassium chloride solutions and have made some modification of the Ives-Swaroopa cell, primarily to simplify the washing and filling of the cell.

II. Experimental

The cell consisted of two antisymmetrical halves joined together by a tube of diameter 1·5 cm and length 5 cm. Each half consisted of a current-carrying arm with a calomel electrode, a central arm and a probe arm with another calomel electrode. The central arm had within it a concentric cylinder which was closed at the lower end and tapered towards the open top ; the current path

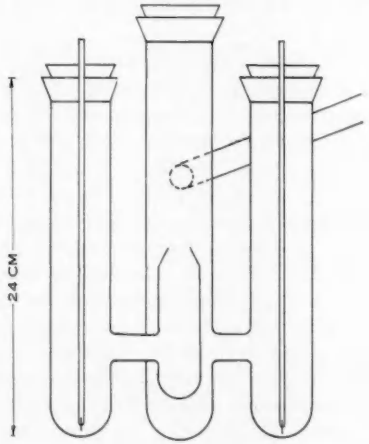

Fig. 1.—The cell (one-half only shown).

was therefore circuitous and there was a large region of zero potential gradient. This inner tube was connected by a side tube to the current-carrying arm (see Fig. 1).

The water used for making the solutions had a specific conductance of the order of 2×10^{-6} ohm^{-1} cm^{-1} ; this was negligibly small compared with the specific conductance of the solutions. Sodium and potassium chloride were recrystallized from A.R. material and dried at 400 °C for long periods. All solutions were made up by weight and volume concentrations calculated from density data in the International Critical Tables (1933). The reversible calomel electrodes were prepared by the method of Hills and Ives (1951). The thermostat liquid was commercial paraffin, " Diala B ". Resistances were measured in terms of the potential across the two probe electrodes determined by a Leeds and Northrup Type K2 potentiometer with current flowing through the cell and in series, through a constant manganin wire resistance (299·9 ohm). Both

the cell and the resistance, the latter in a copper tube, were immersed in the thermostat. The absolute value of the resistance was not needed because the cell was calibrated in terms of an approximately demal potassium chloride solution. The current through the cell and the resistance was kept constant by an electronic circuit essentially similar to that of LeRoy and Gordon (1938).

Viscosities were measured in a conventional Ostwald viscometer with a time of flow of about 850 sec for water at 25 °C and the usual kinematic correction was made by measuring the viscosity of 20 per cent. sucrose at the same temperature.

TABLE 1

EQUIVALENT CONDUCTIVITY OF SODIUM CHLORIDE AND POTASSIUM CHLORIDE SOLUTIONS AT 25 °C

c in mole l^{-1}, Λ in int. ohm^{-1} mole^{-1} cm^2

Sodium Chloride		Potassium Chloride	
c	Λ	c	Λ
0·5018	93·37	0·09534	129·33
1·023	85·64	0·2031	123·68
1·422	80·74	0·3180	121·06
1·747	77·38	0·4902	117·13
2·385	71·36	0·6447	114·80
2·795	67·72	*1·0029*	*111·86*
3·194	64·12	1·646	107·89
3·745	59·63	1·935	106·14
4·284	55·14	2·359	103·28
4·765	51·35	2·865	100·36
5·387	46·82	2·902	100·34
		3·027	99·43
		3·233	98·29
		3·423	96·72
		3·765	94·79
		4·043	93·37

III. RESULTS

Table 1 gives the observed equivalent conductivities of 11 solutions of sodium chloride and 16 solutions of potassium chloride at 25 °C. The measured resistances were converted to equivalent conductivities via the measurements on the 1·0029N KCl solution. The demal solution of Jones and Bradshaw (1933) is 0·9949N at 25 °C and a slight interpolation of a Λ-$\sqrt{c}$ curve gives the equivalent conductivity of the 1·0029N solution as 111·86, only 0·05 unit less than the demal standard; the correction being small can be made with confidence.

Interpolation of the conductivity data of Shedlovsky (1932) gave Λ=129·31 for 0·09534N KCl in good agreement with our value of Λ=129·33. In the dilute solutions, therefore, this D.C. method gives results consistent with the

A.C. method. Our final results indicate that the accuracy of the conductivity data in Table 1 is about 0·05 to 0·1 per cent. Conductivity data at higher concentrations being scanty, a comparison of the results obtained by the two methods cannot be undertaken at this stage. The viscosity results are given in Table 2.

TABLE 2

RELATIVE VISCOSITY OF SODIUM AND POTASSIUM CHLORIDE SOLUTIONS AT 25 °C

Sodium Chloride		Potassium Chloride	
c	η/η°	c	η/η°
0·6589	1·0594	1·1826	0·9976
1·0141	1·0958	2·429	1·018
2·1676	1·2400	2·4895	1·0184
2·419	1·278	2·808	1·031
3·331	1·439	3·535	1·057
3·507	1·472	3·613	1·061
3·5106	1·4746	3·8530	1·0674
4·119	1·609	4·0760	1·0802
4·2333	1·6412		
5·0528	1·8739		
5·205	1·933		

IV. DISCUSSION

The Debye-Hückel-Onsager equation, in a recent elaboration by Falkenhagen, Leist, and Kelbg (1952) has been shown by Robinson and Stokes (1955) to be expressible in the following form:

$$\Lambda=\left[\Lambda^\circ-\frac{B_2\sqrt{c}}{1+B\mathring{a}\sqrt{c}}\right]\left[1-\frac{B_1\sqrt{c}}{1+B\mathring{a}\sqrt{c}}F\right], \quad \ldots\ldots\ldots (1)$$

where

$$F=[e^{\varkappa a(1-\sqrt{q})}-1]/[\varkappa a(1-\sqrt{q})].$$

The way in which conductivity varies with concentration is considered to depend on the effects of electrophoresis and relaxation. In his extension of the work of the earlier authors, Falkenhagen used the complete expression for the electric field and the Eigen-Wicke modification of the Boltzmann statistics. Robinson and Stokes (1955) consider that errors arising out of the need (fundamental as well as practical) to use an approximate expression for the distribution function exceed (or are at least comparable with) the effect of the Eigen-Wicke factor which is omitted from equation (1). Much more important is the introduction of the a term—the distance of closest approach of two ions.

It is not known how viscosity is related to electrophoresis and "relaxation". What is certain from the results so far collected (especially those of the conductivity of solutions whose viscosities increase rapidly with concentration) is that neither of these effects accounts in its entirety for the effect that accompanies a change in viscosity. Wishaw and Stokes (1954) were able to extend

considerably the range of validity of the Falkenhagen equation by making the following modification:

$$\Lambda=\frac{\eta^\circ}{\eta}\left[\Lambda^\circ-\frac{B_2\sqrt{c}}{1+B\mathring{a}\sqrt{c}}\right]\left[1-\frac{B_1\sqrt{c}}{1+B\mathring{a}\sqrt{c}}F\right]. \quad \ldots\ldots\ldots \quad (2)$$

The nomenclature is that of Stokes (1955).

It might be, however, that in addition to introducing the η°/η factor for the effect of changing viscosity on the motion of the ions, we also need to correct the viscosity in the electrophoretic term

$$\Lambda=\frac{\eta^\circ}{\eta}\left[\Lambda^\circ-\frac{\eta^\circ}{\eta}\frac{B_2\sqrt{c}}{1+B\mathring{a}\sqrt{c}}\right]\left[1-\frac{B_1\sqrt{c}}{1+B\mathring{a}\sqrt{c}}F\right]. \quad \ldots\ldots \quad (3)$$

There is a further modification which Falkenhagen and Leist (1954) have published recently

$$\Lambda=\frac{\eta^\circ}{\eta}\Lambda^\circ-\frac{e^2}{3\varepsilon^\circ kT}\left[\frac{\varkappa\Lambda^\circ\eta^\circ}{\eta(1+\varkappa a)}\frac{q}{(1+\sqrt{q})(1+\varkappa a\sqrt{q})}\right]-\frac{Ne^2}{3\pi\eta}\frac{1}{9\times10^{11}}\frac{\varkappa}{1+\varkappa a},$$

which reduces to

$$\Lambda=\frac{\eta^\circ}{\eta}\left[\Lambda^\circ-\frac{B_1\sqrt{c}}{(1+B\mathring{a}\sqrt{c})(1+B\mathring{a}\sqrt{qc})}-\frac{B_2\sqrt{c}}{1+B\mathring{a}\sqrt{c}}\right]. \quad \ldots. \quad (4)$$

Table 3 shows the kind of agreement with experimental data that can be obtained using these four equations.

It may be seen that:

(i) Equation (1), using those values of $\mathring{a}$ which give concordance with data in very dilute solution, does not give even the right order of magnitude for lithium and sodium chloride. The calculated conductivities are too *high* and no adjustment of the $\mathring{a}$ parameter can account for this; solutions of these two salts have viscosities considerably greater than that of the pure solvent. For potassium chloride, however, equation (1) does at least predict the right order of magnitude although the agreement with the experimental data is not good; it may be significant that for this salt (which is only slightly hydrated and the viscosity of its solutions is not very different from that of the pure solvent), the calculated results are too *low*. As the conductivities are of the right order of magnitude, better agreement can be obtained by using a higher $\mathring{a}$ value; $\mathring{a}=4\cdot75$ gives much better agreement but so high a value is not substantiated by any other work.

(ii) Equation (2) works very much better for all three salts; it predicts the conductivities within about three units but the predicted values are all low and the necessary $\mathring{a}$ values seem much too high.

(iii) Equation (3) is even better, the predicted values being within about one conductivity unit of the observed but again the $\mathring{a}$ values are too high.

(iv) Equation (4) gives agreement within, on the average, less than one conductivity unit and the $\mathring{a}$ values are of a more reasonable magnitude.

TABLE 3

OBSERVED AND CALCULATED VALUES OF Λ USING EQUATIONS (1) TO (4) FOR LITHIUM, SODIUM, AND POTASSIUM CHLORIDE

Lithium Chloride $\Lambda^\circ = 115 \cdot 03$

c	$\Lambda_{obs.}$	$\Lambda_{eqn.\ (1)}$ $\dot{a}=4 \cdot 2$	$\Lambda_{eqn.\ (2)}$ $\dot{a}=5 \cdot 2$	$\Lambda_{eqn.\ (3)}$ $\dot{a}=5 \cdot 2$	$\Lambda_{eqn.\ (4)}$ $\dot{a}=4 \cdot 3$
1	73·1	79·1	71·7	74·2	73·8
2	61·7	73·4	58·8	62·8	61·7
3	52·9	70·0	49·2	54·2	52·7
4	44·7	67·4	41·2	46·8	45·1
5	37·8	65·3	34·3	39·9	38·3
6	31·7	63·5	28·3	33·7	32·2
7	26·0	61·9	23·0	28·0	26·7
8	20·9	60·4	18·3	22·8	21·8
9	16·4	59·0	14·3	18·2	17·4
10	13·2	57·7	10·9	14·1	13·5
11	10·4	56·2	8·17	10·5	10·4
12	$8 \cdot 1_4$	55·3	6·03	7·98	7·81
14·04	$5 \cdot 1_1$	52·9	2·95	3·95	4·07

Sodium Chloride $\Lambda^\circ = 126 \cdot 45$

c	$\Lambda_{obs.}$	$\Lambda_{eqn.\ (1)}$ $\dot{a}=3 \cdot 8$	$\Lambda_{eqn.\ (2)}$ $\dot{a}=5 \cdot 0$	$\Lambda_{eqn.\ (3)}$ $\dot{a}=5 \cdot 0$	$\Lambda_{eqn.\ (4)}$ $\dot{a}=4 \cdot 3$
0·5	93·4	93·6	92·9	93·7	94·8
1·0	85·9	89·4	84·1	85·7	87·1
1·5	79·9	83·9	76·4	79·8	81·3
2·0	74·9	81·0	71·3	74·6	75·9
2·5	70·3	78·9	65·8	69·7	70·9
3·0	65·9	77·1	60·6	65·1	66·0
3·5	61·6	75·6	55·7	60·7	61·3
4·0	57·5	74·2	51·0	56·4	56·8
4·5	53·5	73·0	46·7	52·4	52·5
5·0	49·6	72·0	42·2	48·0	48·1

Potassium Chloride $\Lambda^\circ = 149 \cdot 82$

c	$\Lambda_{obs.}$	$\Lambda_{eqn.\ (1)}$ $\dot{a}=3 \cdot 3$	$\Lambda_{eqn.\ (2)}$ $\dot{a}=4 \cdot 75$	$\Lambda_{eqn.\ (3)}$ $\dot{a}=4 \cdot 75$	$\Lambda_{eqn.\ (4)}$ $\dot{a}=3 \cdot 2$
1·0	111·9	105·3	112·4	112·3	111·2
1·5	108·7	100·7	108·5	108·4	107·9
2·0	105·7	97·4	105·0	105·1	105·3
2·5	102·7	94·8	101·6	102·1	102·7
3·0	99·7	92·7	98·3	99·1	100·1
3·5	96·6	90·8	94·9	96·1	97·5
4·0	93·4	89·2	91·6	93·2	95·2

In conclusion, we may note that the agreement between theory and experiment is by no means as good, even in the most favourable example, as we are accustomed to with dilute solutions. Nevertheless, it is clear that the viscosity is the dominant factor in determining the conductance of a concentrated solution.

V. References

Blake, G. G. (1950).—"Conductimetric Analysis at Radio-Frequency." (Chapman and Hall Ltd.: London.)

Eastman, E. D. (1920).—*J. Amer. Chem. Soc.* **42**: 1648.

Falkenhagen, H., and Leist, M. (1954).—*Naturwissenschaften* **41**: 570.

Falkenhagen, H., Leist, M., and Kelbg, G. (1952).—*Ann. Phys.* **11**: 51.

Gunning, H. E., and Gordon, A. R. (1942).—*J. Chem. Phys.* **10**: 126.

Hills, G. J., and Ives, D. J. G. (1951).—*J. Chem. Soc.* **1951**: 318.

International Critical Tables (1929).—Vols. 5, 6. (McGraw-Hill Book Co. Inc.: New York.)

Ives, D. J. G., and Swaroopa, S. (1953).—*Trans. Faraday Soc.* **49**: 788.

Jones, G., and Bollinger, G. M. (1929).—*J. Amer. Chem. Soc.* **51**: 2407.

Jones, G., and Bradshaw, B. C. (1933).—*J. Amer. Chem. Soc.* **55**: 1780.

Jones, G., and Josephs, R. C. (1928).—*J. Amer. Chem. Soc.* **50**: 1049.

Le Roy, D. J., and Gordon, A. R. (1938).—*J. Chem. Phys.* **6**: 398.

Robinson, R. A., and Stokes, R. H. (1955).—"Electrolyte Solutions." (Butterworth Scientific Publications: London.)

Shedlovsky, T. (1932).—*J. Amer. Chem. Soc.* **54**: 1411.

Stokes, R. H. (1955).—*Rev. Pure & Appl. Chem.* **5**: 60.

Wishaw, B. F., and Stokes, R. H. (1954).—*J. Amer. Chem. Soc.* **76**: 2065.

THE ζ-POTENTIAL OF KAOLINITE PARTICLES

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Summary

Electrophoretic mobility measurements, and both conductometric and potentiometric titrations, were carried out on a kaolinite suspension throughout its neutralization by various bases. The concentration of the ionic species present was calculated from the conductometric and potentiometric titrations, and the true ζ-potential calculated from the electrophoretic mobility by Stigter and Mysels's (1955) method. The results indicate that a discontinuity exists in the adsorption of ions in the vicinity of pH 6·5–7·0 causing a considerable increase in the surface charge density of the particles.

I. Introduction

(*a*) *Previous Measurements*

Amongst the previous measurements of ζ-potentials of clay minerals, those of Baver (1929) on Putnam clay are the most interesting since he also measured pH, specific conductivity, and viscosity on the suspensions. In work on kaolinite, no such studies are available, although Coughanour and Utter (1944) report ζ-potentials for kaolinite systems. However, no measurements of the conductivity or pH were made from which data could be obtained to estimate the ionic concentration of the solution. The results of the conductometric titrations of the present study indicate that up to the equivalence point, almost all the added ions of base are removed from the solution so that these data are definitely necessary if the ionic concentration is to be calculated. This ionic concentration is of course essential both for a calculation of the true ζ-potential or of the surface charge density.

(*b*) *Electrophoresis*

Smoluchowski (1914) showed that the electrophoretic mobility per unit field strength of a particle u, moving through a liquid of viscosity η, dielectric constant D, under the influence of a homogeneous electric field, is given by

$$u=\frac{\zeta D}{4\pi\eta}. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

This equation is true for large insulating particles of " easy shape " (Abramson, Moyer, and Gorin 1942), or for large cylinders moving with their axes perpendicular to the electrodes, provided the radius of curvature at all points on

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the surface is much greater than the thickness of the double layer. Hückel (1924) obtained the equation

$$u=\frac{\zeta D}{6\pi\eta}. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Henry (1931) later showed that the concepts of these authors differed in the consideration of the geometry of the applied field. Smoluchowski considered the particles to be insulating and took the deformation of the applied field by the particle explicitly into account, whereas Hückel simply assumed that the value and direction of the electric field were constant throughout the whole system. Henry's solution is valid for any ratio between the thickness of the double layer and particle dimension, although limited to spherical particles and to cylinders with their axes parallel or perpendicular to the applied field. He obtains

$$u=\frac{\zeta D}{6\pi\eta} \cdot f(\varkappa a), \quad \ldots\ldots\ldots\ldots\ldots \quad (3)$$

where $f(\varkappa a)$ is a complicated function of $\varkappa$, and $f(\varkappa a)$ is plotted in Henry's paper. For low values of $\varkappa a$, that is, small spheres (although large enough to obey Stokes's law) $f(\varkappa a) \propto 1$ and Hückel's equation holds, whilst for large spheres $f(\varkappa a) \propto 1 \cdot 5$ and the Smoluchowski equation holds.

Various corrections are necessary in certain cases:

(i) *For Finite Size of Ions.*—Gorin (1939; see also Abramson, Moyer, and Gorin 1942) calculated the corrections necessary to allow for the finite ionic sizes, however, except with very high ionic strengths, or extremely small particles, these corrections are negligible.

(ii) *For Surface Conductance.*—Booth (1948) and Henry (1948) have considered the corrections necessary when the surface conductivity is taken into account. Henry shows that, for an insulating particle

$$u=\frac{D\zeta}{4\pi\eta} \cdot \frac{\lambda_0}{\lambda_0+(\lambda_s/a)}, \quad \ldots\ldots\ldots\ldots\ldots \quad (4)$$

where λ_0 = specific conductivity of the medium,
λ_s = surface conductivity of particles,
a = particle radius.

If the surface conductivity has a normal value, that is, approximately equal to that calculated from the true ζ-potential, then the surface conductivity correction will be included in that for the influence of relaxation. It has been shown (Street 1956*a*) that these kaolinite particles do exhibit normal surface conductivity.

(iii) *For the Effect of Relaxation.*—This effect, arising from the deformation of the double layer, has a retarding influence on the electrophoresis. In an applied field the charge of the diffuse double layer is displaced in a direction opposite to the movement of the particle, this not only retarding the electrophoresis by its movement, but also, by the resulting dissymmetry of the double layer, sets up

a retarding potential difference. Corrections have been suggested by several authors (Overbeek 1943, 1946; Booth 1950). Overbeek points out (Kruyt 1952) that, *inter alia*, the relaxation effect may give important corrections if the ζ-potential is not very small for values of κa from 0·1–100. This means that very often conclusions as to the value of ζ derived from the electrophoretic measurements are open to serious doubt. The equation developed by Stigter and Mysels (1955) is applicable to the present data for the univalent cations and includes the Henry and the relaxation corrections. Using their notation this is

$$\Phi_0=\frac{u'}{X_1^*}-\frac{X_3^*+Y_3^*+q_3Z_3^*}{X_1^*}\left(\frac{u'}{X_1^*}\right)^3-\frac{q_4^*Z_4^*}{X_1^*}\left(\frac{u'}{X_1^*}\right)^4, \quad \ldots\ldots \quad (5)$$

where $\Phi_0=\varepsilon\zeta/kT$,
$u'=(6\pi\eta\varepsilon/DkT)u$,
u =mobility (μ/sec/V/cm),

X^*, Y^*, and Z^* are available in Henry (1931), and $q^*/6$ in Overbeek (1943).

II. Experimental

(*a*) *Electrophoretic Mobility*

The cell used was constructed from a Pyrex capillary with flats ground and polished at right angles to each other; two B10 sockets were fitted at the ends so that either a funnel could be fitted at one end and a siphon at the other end, or else glass plugs with fine bores fitted so that the cell could be completely filled with the solution under investigation and still preserve the essentials of a closed cell. Tungsten electrodes were used (see below) and the cell cemented to a glass flat to facilitate positioning on the platform. The normal stage of the Beck microscope used was removed, and a special brass stage constructed which could be fitted to the substage condenser mount, thus permitting both vertical and horizontal movement of the platform. Since measurements in the cell are restricted to concentrations not greater than 10^{-3}N, gas evolution does not present difficulties; also, at the low current densities used, polarization of the Tungsten electrodes may be neglected, so that the overvoltage will be negligible in comparison with the applied potential (Creighton and Koehler 1943).

In order that true electrophoretic mobilities, independent of any electro-osmotic effect, may be measured, it is necessary that the correct (actually annular) layer be observed. Provided the cell is correctly centred, then this level is located by focusing the correct distance from the roof of the cell. The cell was centred by the method of van Gils and Kruyt (1936), and allowance made for optical errors in focusing by the methods of Hukki, Palomaki, and Orivuori (1952). The bore of the capillary used was accurately determined by calibration with mercury threads.

Using Hukki, Palomaki, and Orivuori's formula, the focusing distances were calculated for distances from the roof other than the stationary level and the velocities of a clay suspension determined at the various levels. Figure 1 shows that a plot of r^2/r_0^2 (where r=vertical distance from cell bore to particle level, r_0=radius of capillary) *against* mobility gives a straight line, and that the

measured value for the stationary ($0 \cdot 293D$) level falls on this line at $r^2/r_0^2 = 0 \cdot 5$; thus the stationary level being observed is apparently the true level (Munro and Sexsmith 1953).

The potential drop across the cell was measured (see also van Gils and Kruyt 1936 ; Hukki, Palomaki, and Orivuori 1952 ; Munro and Sexsmith 1953) rather than the current through it. The circuit used is as shown in Figure 2, the D.C. potential was supplied by radio B batteries (90 V), the values of C and D are each 10,000 Ω. The DPDT switch allows the polarity to be reversed without affecting the reading on the voltmeter (Tinsley variable range Voltammeter Type 4237A).

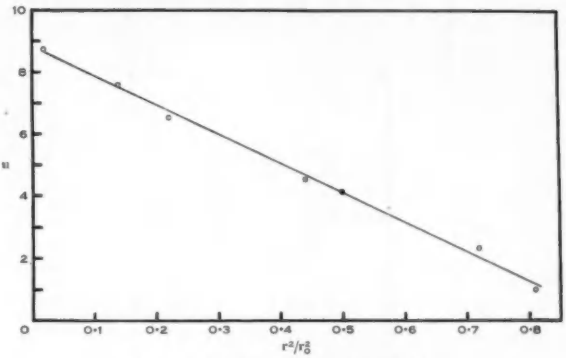

Fig. 1.—r^2/r_0^2-electrophoretic mobility.

The length l_c of the cell (Fig. 3) by which the measured potential must be divided in order to give the potential drop (in V/cm) is neither the length l of the capillary section, nor the distance $L+l$ between the electrodes, but is given by $l_c = aK'X$ (Street 1956*b*) ; where X = measured resistance of the cell when filled with liquid of conductivity K', a = cross-sectional area of the capillary section. For the cell used $a = 0 \cdot 0366$ cm² and $l_c = 6 \cdot 69$ cm.

The illumination system used was essentially that provided for a Zeiss slit ultramicroscope (Chamot and Mason 1944, p. 213) using a D.C. arc as the light source. The light was passed through a cooling flask of saturated alum solution, then a narrow beam from the slit was focused, by means of a 16 mm objective, onto the liquid in the capillary of the cell.

In a series of measurements using increasing concentrations of the same electrolyte it was possible to rinse out the cell with successive amounts of the electrolyte using the funnel and siphon. The eyepiece of the microscope was fitted with a scale of $7 \cdot 6\mu$/scale division (at the magnification used), and the motion in each direction past the scale of at least 10 particles was timed by means of a stop watch. Since an objective of 16 mm was used, an eyepiece of 7×, and the tube length of the microscope had been increased to about 160 mm with the addition of an angular eyepiece attachment, the magnification was of the order of 70×.

(b) Preparation of Kaolinite

The kaolinite used was obtained from Nilsen Porcelain Pty. Ltd. and is a pure kaolin mined at Egerton, Victoria (Bain and Spencer-Jones 1953).

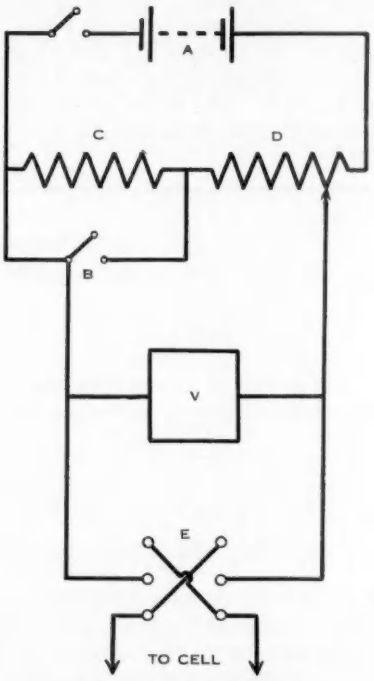

Fig. 2.—Wiring diagram.

An analysis of the untreated material gave the data presented in Table 1. A bulk sample of 50 lb was plunged in a pilot plant at the Nilsen Porcelain Co.'s works, and the resulting thick slurry treated in the laboratory with sufficient

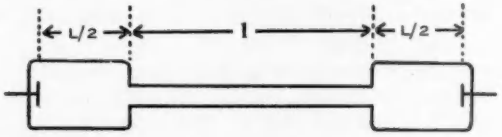

Fig. 3.—Diagrammatic representation of electrophoresis cell.

sodium hydroxide to deflocculate it. It was then separated by repeated settling into a relatively narrow range of particle sizes. This fraction was filtered on a large Buchner funnel, and, after dispersion in distilled water, was treated with 1 per cent. (on a clay basis) of 30 per cent. hydrogen peroxide to remove organic

matter (Johnson and Norton 1941). The reaction was allowed to proceed with intermittent stirring for 1 week, after which the clay was again dispersed with sodium hydroxide and washed by centrifuging until electrolyte free. The sample was then treated with successive amounts of 0·05N hydrochloric acid to convert it to the hydrogen form. The kaolin was again thoroughly washed by centrifugation until the filtrate conductivity was of the order of $0·5 \times 10^{-5}$ ohm^{-1} cm^{-1}. At no time during the preparation was the clay allowed to dry out. A stock suspension of high sp. gr. hydrogen clay was kept

TABLE 1

KAOLIN ANALYSIS

Components	Egerton Kaolin	Kaolinite (theoretical)
Al_2O_3	39·27	39·50
SiO_2	45·41	46·50
Fe_2O_3	0·49	—
TiO_2	0·35	—
CaO	0·37	—
MgO	Trace	—
K_2O	0·35	—
Na_2O	0·33	—
H_2O	13·68	14·00
Total	100·25	100·00

in Jena glass bottles and the slurries for the determination of viscosity (Street 1956*b*), or of ζ-potential, were diluted from these stocks. From the stage of the preparation of the hydrogen clay onwards double-distilled water alone was used, the second distillation being in an all-Pyrex still from alkaline permanganate solution.

(*c*) *Preparation of Suspension*

Since it was desired to conduct subsequent rheological measurements on a constant volume concentration, suspension was always diluted to sp. gr. =1·03 (i.e. a volume concentration of 0·0194, the kaolin density having been determined as 2·55). The procedure adopted was to make up a stock solution of the electrolyte being used, then to add slurry, electrolyte solution, and water, in volumes such that the electrolyte concentration would be that desired, and the sp. gr. of the suspension 1·03. The prepared suspensions were stored (with intermittent shaking) in Pyrex ground-glass stoppered flasks for 1 week before measurements were taken.*

* Tests indicated that the mobilities always reached a constant value by 24 hr after the addition of any base to the hydrogen clay; in fact, in most cases equilibrium was almost instantaneous. However, all samples were let stand for 1 week to ensure complete reaction.

In determining the electrophoretic mobility the suspension was centrifuged and a volume of the original suspension, sufficient to give a volume concentration of 0·0001, was added to some of the filtrate and the mobility determined on this diluted suspension. Experiments had shown that there was virtually no variation in mobility when measured with the volume concentration between 0·00002 and 0·0005.

(*d*) *Conductometric Titration*

Conductivity measurements were carried out on the suspension (sp. gr. =1·03) equilibrated to 25 °C in a thermostat; after centrifugation the conductivity of the filtrate (at 25 °C) was determined. The results of the conductivity measurements with the various bases are shown in Figure 4.

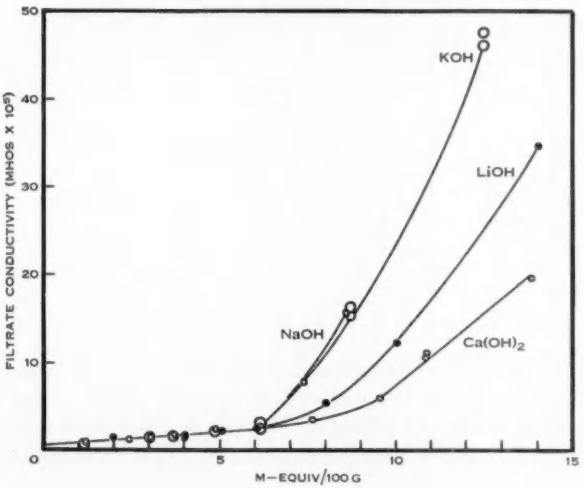

Fig. 4.—Conductometric titrations.

Since it has been suggested that the clay lattice is readily attacked by even the dilute hydrochloric acid used to convert the kaolinite to the hydrogen form, a series of tests were undertaken. The hydrogen clay was titrated conductometrically to the sodium hydroxide end-point, reconverted to hydrogen clay with 0·05N hydrochloric acid, retitrated, and the whole process repeated 10 times; the differences found between the titrations were within the possible experimental error and would suggest that in fact very little breakdown of the kaolinite lattice has occurred in this treatment.

(*e*) *Potentiometric Titration*

The potentiometric titrations were carried out on sp. gr. =1·03 suspensions of the hydrogen kaolinite using a Cambridge pH meter and a glass electrode.

III. Results

Figure 5 gives the results of the electrophoretic mobilities of these systems.

The electrophoretic mobility of clay treated with lithium hydroxide shows a moderate and approximately uniform increase up to about 4 m-equiv/100 g, and thereafter increases much more rapidly. The mobility with sodium hydroxide increases up to about 5 m-equiv. rather more rapidly than that for lithium hydroxide. The initial rise in mobility for potassium hydroxide is even steeper than for sodium hydroxide and is followed by a step in the curve at 5 m-equiv. before the steeper rise towards the equivalence point.

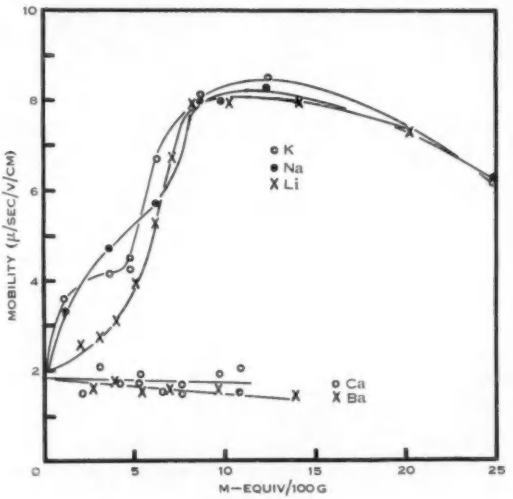

Fig. 5.—Electrophoretic mobilities.

The titration curves of the clay, that is, the measured pH's of the suspensions of 1·03 sp. gr. hydrogen kaolinite after addition of the noted amount of each hydroxide (calculated on a clay basis) are given in Figure 6. It is not surprising that the pH curves do not parallel the mobility curves, since the change in pH is due to removal of hydrogen ions from the bulk solution, whereas the change in mobility gives a measure of the change in cation dissociated from the surface of the clay particle ; some of the exchanged cations may be firmly bound to the clay surface, and some dissociated from it contributing to the double layer potential.

The conductometric titration curves (Fig. 4) show inflexions as indicated in Table 2. There is no really significant difference between the values of the point of inflexion of the conductometric curves.

The exchange capacity of the clay was also determined by the method of equilibration of hydrogen clay with 1N metal chloride solution for 24 hr, followed by centrifugation, washing with 1N chloride, and titration with N/50 baryta

solution (Table 3). The exchange capacities as taken from the full titration curve at pH=7 are shown in Table 4.

These results agree with those of Mukherjee, Mitra, and Mitra (1943) who found that the base exchange capacity for a kaolin at pH=7 was

$$NaOH : Ba(OH)_2 : Ca(OH_2) : : 1 \cdot 0 : 1 \cdot 8 : 2 \cdot 2,$$

the results of this study give the ratios $1 \cdot 0 : 2 \cdot 0 : 2 \cdot 2$.

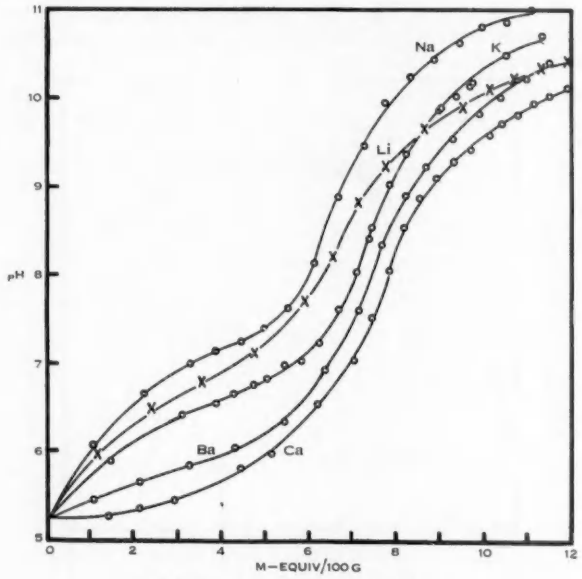

Fig. 6.—Potentiometric titrations.

Results from the plots of the pH and conductometric titrations have been used to calculate the concentrations of the various ions present in the intermicellar liquid in these suspensions. The concentration of H^+ and OH^- was obtained from the pH curves and from these the conductivity to be expected

TABLE 2

POINTS OF INFLEXION OF CONDUCTOMETRIC TITRATIONS (M-EQUIV/100 G)

LiOH	NaOH	KOH	$Ca(OH)_2$	$Ba(OH)_2$
6·1	6·2	6·1	6·0	6·0

was determined (using normal ionic mobilities) from either H^+ plus HCO_3^- or OH^- plus the cation concerned. The measured conductances were always greater than the calculated and were ascribed to the cation concerned plus HCO_3^-, the concentration of these was calculated using normal ionic mobilities.

Figure 7 gives the value of $\varkappa$, that is, the reciprocal of the double layer thickness as calculated from the expression $\varkappa = 0 \cdot 327 \times 10^8 \sqrt{\mu}$ (μ = ionic strength) and using these ionic concentrations. These values of $\varkappa$ have been used in calculating $\varkappa a$ to insert in the equation for the calculation of the true ζ-potential from the

TABLE 3

EXCHANGE CAPACITY WITH NEUTRAL CHLORIDE (M-EQUIV/100 G)

Salt	Exchange Capacity	Salt	Exchange Capacity
LiCl	4·26	$CaCl_2$	5·11
NaCl	4·69	$MgCl_2$	5·34
KCl	4·83	$BaCl_2$	5·26

mobility data assuming that these clay particles behave as spheres of radius $0 \cdot 15 \times 10^{-4}$ cm. This value for the radius a was calculated from the surface area determined by the B.E.T. method ($17 \cdot 8 \times 10^4$ cm^2/g) and assuming an approximately monodisperse system. Then, since the axial ratios of these particles is between 11 and 12 (Street 1956*b*) the volume of one particle is approxi-

TABLE 4

EXCHANGE CAPACITY AT PH = 7 (M-EQUIV/100 G)

Base	Exchange Capacity	Base	Exchange Capacity
LiOH	4·4	$Ca(OH)_2$ ..	7·0
NaOH	3·2	$Mg(OH)_2$ ..	—
KOH	5·7	$Ba(OH)_2$ ..	6·5

mately $13 \cdot 5 \times 10^{-15}$ c.c. and the radius of a sphere of equal volume is approximately $0 \cdot 15 \times 10^{-4}$ cm.

Figure 8 gives the results of the true ζ as calculated in this manner. The ζ-potentials of the alkali hydroxides were calculated by the method of Stigter and Mysels (1955), and those of the alkaline earths using Henry's (1931) equation.

IV. DISCUSSION

The experimentally determined dissociation constants of the metal hydroxides (Davies 1951; Gimblet and Monk 1954) in aqueous solutions are recorded in Table 5, together with the initial slopes of the ζ-potential and electrophoretic mobility curves. It is believed that the values of these dissociation constants are determined by the strength of the electrostatic interaction between cation and hydroxyl ion, which is in turn a function of the closeness of approach of the two ions as shown by their crystal radii. By analogy the degree of dissociation of the alkali metal kaolinites can be expected to be in the order K > Na > Li, which is in fact the order of the initial slopes of both the

ζ-potential and the electrophoretic mobility curves. Any increase in ζ-potential for the first addition of alkali to the clay is most likely to be due to dissociation of those surface groups of the kaolinite which have taken part in cation exchange with the alkali, hence the observed increase in slope with increase in dissociation constant. The low initial slope of the Li-clay is also probably related to the result of Keenan, Mooney, and Wood (1950) who determine by a B.E.T. plot

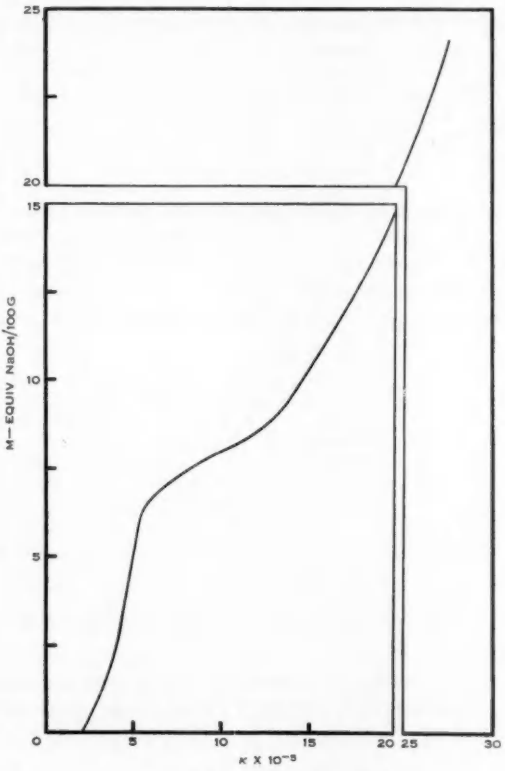

Fig. 7.—κ-added sodium hydroxide.

(using water vapour) of Li-kaolinite that no water is associated with each ion of Li^+, in contrast to 2·4 for Na^+ and 1·6 for K^+ (molecules per ion). They suggest that, since the exchangeable ions will take up positions determined by the equilibrium between the forces of hydration and the forces tending to pull the ion into the surface layer, this result is explained by the small size of the Li^+ ion allowing it to sink well into the surface layer where hydration is sterically hindered. The low initial slope of the ζ-potential curve for the calcium and barium kaolinites is in accord with the small dissociation constants of their

hydroxides. After a very slight increase the values again decrease at about the equivalence point where the increasing concentration of electrolyte causes compression of the double layer and thus decrease in the ζ-potential.

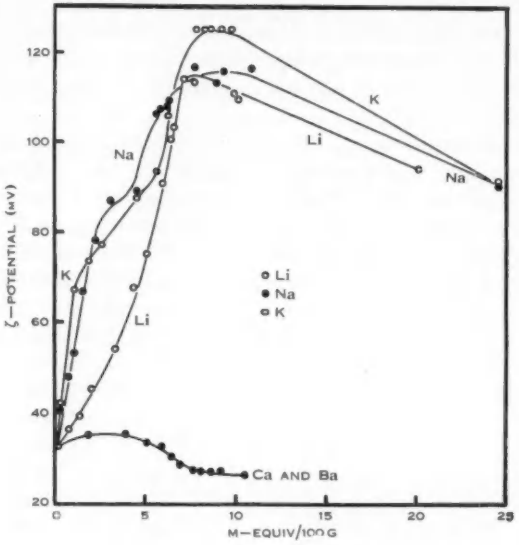

Fig. 8.—True ζ-potentials.

The evidence of both the ζ-potential curves, and the yield value curves, as will be discussed in a subsequent paper, support the suggestion by van Olphen (1950, 1951), and of Samson (1953) and Schofield and Samson (1954) of the

TABLE 5

INITIAL SLOPE ζ AND u

Cation	Initial Slope, ζ (ζ/m-equiv)	Initial Slope, u (u/m-equiv)	Dissociation Constant (hydroxyl)		Sum of Crystal Radii
			Davies (1951)	Gimblet and Monk (1954)	
Li ..	8	0·27	1·2	0·7	2·0
Na ..	17	1·07	5	6	2·35
K	26	1·30	High	6	2·73
Mg	—	—	0·0026	—	2·05
Ca	1·4	Neg.	0·05	—	2·39
Ba ..	1·4	Neg.	0·23	—	2·75

existence of positively charged edges and negatively charged faces on the kaolinite particles. There have been several suggestions regarding the formation of the exchange sites on kaolinite particles. Mukherjee and Gangully (1950)

suggest that there is an almost complete absence of isomorphous replacement, and that the exchange sites are on the exposed OH groups of the hydroxyl surface of the crystal, and that the exchange capacity is due to dissociation of these OH groups.

Mitra (1941–42) reports two inflexion points on the titration curve, and suggests that the subsurface OH groups surrounded by O are able to exchange H at higher pH. The present titration curves do not show evidence on this diabasic character.

Grim (1953) states, "In the Kaolinite and Halloysite minerals broken bonds are the major cause of exchange capacity", that is, he suggests that broken bonds around the edges of the SiO_2-Al_2O_3 units give rise to unsatisfied charges which are balanced by adsorbed cations, these broken bonds being on the vertical planes parallel to the C-axis. This contention was earlier suggested by Hendricks (1945), who quotes in evidence the results of Johnson and Lawrence (1942) on the variation of cation exchange with particle size. Samson (1953) has re-examined the data of Johnson and Lawrence and points out that the exchange capacity was determined from the quantity of NaOH required to deflocculate samples of electro-dialysed kaolin, but the points of equivalence were not taken at exactly comparable points on each curve. On replotting, the relationship is seen to be only very approximately linear.

An alternative suggestion by Schofield and Samson (1953) is that the exchange capacity of the kaolinite is due to isomorphous replacement within the clay lattice in the same manner as in montmorillonite. They point out that only 1 silicon in 400 needs to be replaced by aluminium to give an exchange capacity of 2m-equiv/100 g, and that it would be very difficult to prove or disprove so small a replacement by chemical analysis. They further point out that Hendricks (1945) neglected the effect of pH in deducing from the crystal structure that oxygens at the edge of a silicon-oxygen sheet, being bonded to only 1 silicon will each carry a negative charge, and that the pair of oxygens at the edge of the gibbsite layers (each bonded to hydrogen or silicon, and, by a half-bond, to one aluminium) will each carry one-half negative charge. Hendricks's model supposes each 33 $Å^2$ of edge face will carry two negative charges.

However, these edge oxygens can form additional bonds to hydrogen ions, and under neutral and acid conditions will be combined with hydrogen ions to form uncharged hydroxyls. Schofield and Samson have shown that positive adsorption of chloride ions occurs, thus suggesting the existence of these positive areas. Samson also shows that positive adsorption of Cl^- is greatest under strongly acid conditions and decreases, at first rather gradually, as the pH is increased. The adsorption becomes zero at about pH=6 and is thereafter negative (i.e. due to expulsion of ions from the double layer). A constant value of the negative adsorption is reached at about pH=7·5.

It is also of interest to consider the facts pointed out by Keenan, Mooney, and Wood, namely, that if the edges alone are the exchange sites, then the exchangeable ions on kaolinite are concentrated much more closely than on

montmorillonite ; in fact, the larger monovalent cations would be almost close packed on the surface. If the ions are scattered over all the surface then their concentration is very little greater than that for montmorillonite. There is probably a rough linear relationship between the exchange capacity and the surface area, but this may well be explained on the basis that those crystals with more isomorphous replacements would be more inhibited in their growth.

In the face of all the evidence it seems reasonable to suggest that the base exchange capacity of the kaolinite in neutral solutions is due to isomorphous replacements in the lattice and that in alkaline solutions (or following adsorption of some polyvalent anion) the edge faces become negatively charged and also take part in the exchange reactions. Perhaps a clearer picture of the discontinuity in the adsorption of ions on the clay surface at or about the equivalence point is shown by the surface charge density curves depicted in Figure 9. The values for the divalent ions were calculated from

$$\sigma=\sqrt{\left(\frac{DkTn_{1(2)}Z_{1(2)}}{2\pi}\right)\left\{\frac{1}{Z_1}(e^{-Z_1\varepsilon\zeta/kT}-1)-\frac{1}{Z_2}(e^{-Z_2\varepsilon\zeta/kT}-1)\right\}}, \quad \ldots\ (6)$$

where σ=surface charge density (e.s.u./cm^2),
D=dielectric constant,
k=Boltzmann's constant,
T=absolute temperature,
$n_{1(2)}$=concentration ions 1,2 (ions/cm^3),
$Z_{1(2)}$=valency of ions 1, 2,
ζ=ζ-potential,
ε=electronic charge.

In the case of uni-univalent electrolytes this may be simplified to

$$\sigma=2\alpha\sqrt{c}\ \sinh\frac{\zeta}{2\beta}, \quad \ldots\ (7)$$

where α=17,650,
β=0·025 (V),
c=concentration (mol/l).

These equations were used with the ionic concentrations obtained from the pH and conductivity measurements as already described.

It can be seen that, as the H^+ on the clay surface are replaced by alkali ions (the salts being more strongly dissociated) the surface charge density gradually increases to a concentration of about 6 m-equiv/100 g, at which point the edges change in character from positive to negative and there is a sudden large increase in the surface charge density. This rapid increase is not necessarily entirely due to neutralization of the positive edge charges ; it seems likely that the 001 faces themselves of the platelets adsorb OH^- and thus contribute to the large increase in the surface charge density, the cations remaining as counter ions in the diffuse layer. It is interesting to observe that the value reached by the surface charge density is also in the order K>Na>Li. The curve for calcium and barium kaolinites shows virtually constant surface charge

density up to the equivalence point; beyond it, it shows an increase similar to that for univalent cations but of much less magnitude, the calcium and barium kaolinites presumably being but little dissociated.

It has been shown (Johansen 1955) that the isoelectric point of Al_2O_3-SiO_2 mixtures vary according to their composition; if the isoelectric point of the kaolin edges is the same as the appropriate composition in these results, then an isoelectric point of about pH=6·5 could be expected. Johansen also

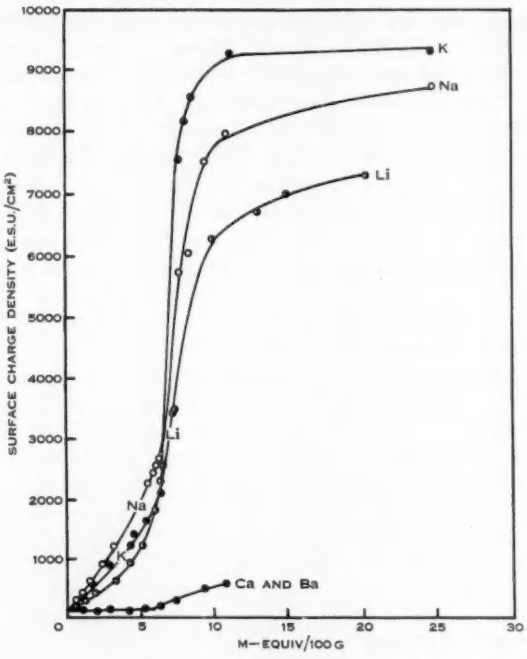

Fig. 9.—Surface charge densities.

determined the effect of pH on the electrophoretic mobility of an alumina silica catalyst of 45·9 per cent. Al_2O_3, that is, fairly close to the composition of a kaolinite. The shape of this curve is very similar to that of Schofield and Samson for Cl^- adsorption against pH and lends further support to the hypothesis of positive edges at low pH.

Schofield (1939) has suggested that there are clays (unspecified mineralogy) which do exhibit an isoelectric point and are positively charged below some particular pH. The fact that kaolinite has never been shown to exhibit a positive potential by decreasing the pH is due to the fact that the negative charge of the 001 face is always greater than the positive charge of the edge face. Figure 10 shows the results of electrophoretic measurements on this kaolin at increasing

acid concentration; it is not possible to carry the measurements to higher concentrations of acid without very considerable modifications of the apparatus. If an isoelectric point exists it is at an extremely low pH and its location must await further investigation.

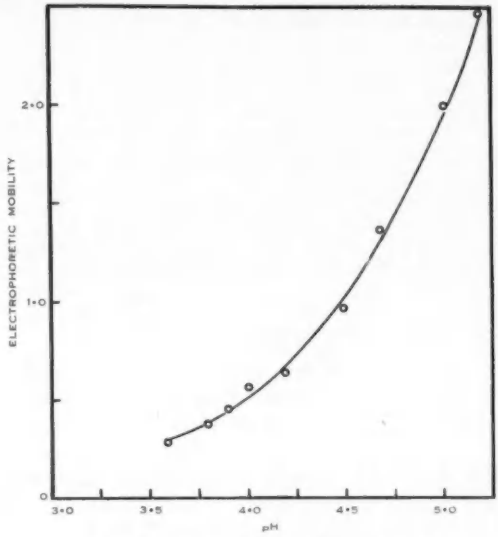

Fig. 10.—pH-electrophoretic mobility.

V. References

Abramson, H. A., Moyer, L. S., and Gorin, M. H. (1942).—"Electrophoresis of Proteins." (Reinhold Publ. Co.: New York.)

Bain, A. D. N., and Spencer-Jones, D. (1953).—*Min. Geol. J.* **5**: 30.

Baver, L. D. (1929).—Res. Bull. Mo. Agric. Exp. Sta. No. 129.

Booth, F. (1948).—*Trans. Faraday Soc.* **44**: 955.

Booth, F. (1950).—*Proc. Roy. Soc.* A **203**: 514.

Chamot, E. M., and Mason, C. W. (1944).—"Handbook of Chemical Microscopy." Vol. 1. (John Wiley & Sons: New York.)

Coughanour, W. L., and Utter, J. L. (1944).—*J. Amer. Ceram. Soc.* **27**: 116.

Creighton, H., and Kohler, W. A. (1943).—"Electrochemistry." Vol. 1. (John Wiley & Sons: New York.)

Davies, C. W. (1951).—*J. Chem. Soc.* **1951**: 1256.

van Gils, G. E., and Kruyt, H. R. (1936).—*Kolloidbeihefte* **45**: 60.

Gimblet, F. G. R., and Monk, C. B. (1954).—*Trans. Faraday Soc.* **50**: 965.

Gorin, M. H. (1939).—*J. Chem. Phys.* **7**: 405.

Gratz, L., and Smoluchowski, M. (1921).—"Graetz Handbuch der Elektrizitat und des Magnetismus." Vol. 2. (J. A. Barth: Liepzig.)

Grim, R. E. (1953).—"Clay Mineralogy." (McGraw-Hill Book Co.: New York.)

Hendricks, S. B. (1945).—*Industr. Engng. Chem.* **37**: 625.

Henry, D. C. (1931).—*Proc. Roy. Soc.* A **133**: 106.

Henry, D. C. (1948).—*Trans. Faraday Soc.* **44**: 1021.

Hückel, E. (1924).—*Phys. Z.* **25**: 204.

HUKKI, R. T., PALOMAKI, A., and ORIVUORI, E. (1952).—*Acta Chem. Fenn.* **25**: 42.
JOHANSEN, P. (1955).—Thesis, University of Melbourne.
JOHNSON, A. L., and LAWRENCE, W. G. (1942).—*J. Amer. Ceram. Soc.* **25**: 344.
JOHNSON, A. L., and NORTON, F. H. (1941).—*J. Amer. Ceram. Soc.* **24**: 189.
KEENAN, A. G., MOONEY, R. W., and WOOD, L. A. (1951).—*J. Phys. Chem.* **55**: 1462.
KRUYT, H. R. (1952).—"Colloid Science." Vol. 1. (Elsevier Publ. Co. Inc.: Amsterdam.)
MITRA, R. P. (1941-42).—*Bull. Soc. Soil Sci.* **4**: 41.
MUKHERJEE, S. K., and GANGULLY, A. K. (1952).—*Indian J. Phys.* **24**: 233.
MUKHERJEE, J. N., MITRA, R. P., and MITRA, K. D. (1943).—*J. Phys. Chem.* **47**: 543.
MUNRO, L. A., and SEXSMITH, F. H. (1953).—*Canad. J. Chem.* **31**: 287.
VAN OLPHEN, H. (1950).—*Rec. Trav. Chim.* **69**: 1308.
VAN OLPHEN, H. (1951).—*Disc. Faraday Soc.* **11**: 82.
OVERBEEK, J. TH. G. (1943).—*Kolloidbeihefte* **54**: 287.
OVERBEEK, J. TH. G. (1946).—*Philips Res. Rep.* **1**: 315.
SAMSON, H. R. (1953).—Thesis, University of London.
SCHOFIELD, R. K. (1939).—*Soils & Fert. (Imp. Bur. Soil Sci.)* **2**: 1.
SCHOFIELD, R. K., and SAMSON, H. R. (1954).—*Disc. Faraday Soc.* **18**: 135.
SMOLUCHOWSKI, M. (1914).—"Graetz Handbuch der Elektrizitat und des Magnetismus." Vol. 2. (J. A. Barth: Liepzig.)
STIGTER, D., and MYSELS, K. J. (1955).—*J. Phys. Chem.* **59**: 45.
STREET, N. (1956*a*).—*Aust. J. Chem.* **9**: 467 .
STREET, N. (1956*b*).—Thesis, University of Melbourne.

THE RHEOLOGY OF KAOLINITE SUSPENSIONS

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Summary

The rheological properties of constant volume concentration kaolinite suspensions were measured and compared with ζ-potential measurements already made. The correlation is good and the results are interpreted on the basis of the existence of both positive and negative areas on the clay surfaces linking the particles together in "bipolar" flocculation; these floccules uncoil with increasing neutralization of the H-clay, then at the equivalence point the positive areas are neutralized, the clay suspension deflocculated and the viscosity considerably reduced.

I. INTRODUCTION

The rheological properties of suspensions of the clay minerals are of importance in several fields, of which those of drilling mud and ceramics are perhaps the best known. The early studies (Baver 1929) were concerned with Putman clay; other studies are listed by Marshall (1948). In much of this early work the viscosity was measured with an Ostwald flow type viscometer, the viscosity of a clay suspension evidently being assumed Newtonian; Baver attempted to correlate his viscosity results with those calculated from the measured ζ-potentials using the Smoluchowski (1916) equation. Few workers since Baver have closely correlated the measured viscosities with measured ζ-potentials, for example, in that of Johnson and Norton (1944) no ζ-potentials are actually measured, but assumptions are made regarding their magnitude on the basis of pH measurements. A further criticism of this work is that the effect of the ionic strength of the electrolyte solution on the dielectric constant is held to be of significance in increasing the ζ-potential, whilst the effect on the double layer thickness is ignored. The work of Collie, Hasted, and Ritson (1948) on the effect of ionic strength on dielectric constant shows that,

$$D=D_w+2\bar{\delta}C, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where D=dielectric constant of solution,
D_w=dielectric constant of water,
C=concentration of electrolyte (mol/l),
$\bar{\delta}$=constant for the particular electrolyte.

Since $\bar{\delta}$ for NaOH is $-10{\cdot}5$, and, in general, is between -7 and -15, it can be seen that, in the range of concentration over which the viscosity shows a rapid change (Street and Buchanan 1956), the effect of the decrease in double layer thickness (giving decreased ζ) is much greater than the effect of the dielectric

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constant (giving increased ζ). There seems to be some confusion in the Johnson and Norton paper between ζ-potential and charge.

The review of Holdridge (1952) illustrates the confused thought extant on this problem; there is no clear idea of the mechanism of deflocculation, and high ζ-potential and high particle hydration are equated. Thus a high viscosity is expected at high ζ because of particle hydration, nevertheless—"Alkali addition, however, causes reduced viscosity although the ζ-potential is increased. The apparent anomaly is due to the deflocculating action of the alkali . . . ". In order to explain the facts the release of mechanically-held water must be postulated as the major factor in viscosity reduction.

Schofield and Samson (1954) recognized the existence of both positive and negative areas on the particles, that is, they introduced the idea of "bipolar" flocculation, however, in their work they dealt only with that portion of the neutralization curve beyond the equivalence point, and they did not measure

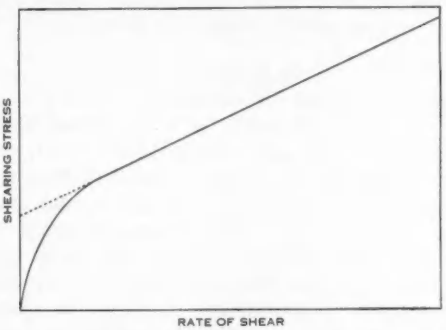

Fig. 1.—Typical shearing stress-rate of shear plot for anomalous flow.

ζ-potentials. To follow the variation of the rheological properties of uniform dilute kaolinite suspensions, and to correlate them with the electrokinetic potential the procedures outlined in the present paper have been adopted.

Since all except the most dilute of kaolinite suspensions exhibit anomalous, that is, non-Newtonian viscosity, it is essential to measure the rheological properties on an instrument capable of giving results at various, and known, rates of shear. If the measurements are obtained with the aid of a concentric cylinder (Couette) type of instrument, then on a plot of rate of shear *against* shearing stress, the yield value may be obtained from the intercept on the shearing stress axis, and the plastic viscosity from the slope of the curve.

The yield value may be considered a measure of the interparticulate forces in a dilute suspension, and is a parameter of the "residual flocculation" in that suspension (de Waele and Lewis 1953).

The plastic viscosity is probably largely determined by the size and shape of the flow units, and to a certain extent by the ζ-potential in obedience to the electroviscous equations of either Smoluchowski (1916), or Booth (1950).

It is possible that no true yield value is actually exhibited by these suspensions, so that the true flow curve is given by the continuous line in Figure 1, if so then it becomes possible to describe the apparent viscosity in terms of η_0 (viscosity at " zero " shear) and η_∞ (viscosity at " infinite " shear). However, in the present paper the term yield value (f) will be retained since it has been shown that there is little difference between the behaviour of f or η_0 in these suspensions.

II. Experimental

A rotational viscometer the rate of shear of which could be varied at will was required for this work. The instrument constructed had a cup and bob with dimensions (in mm) similar to those suggested by van Olphen (1950), namely, diameter of bob=39·6 ; diameter of cup=43·8 ; height of bob=44·7 ; height

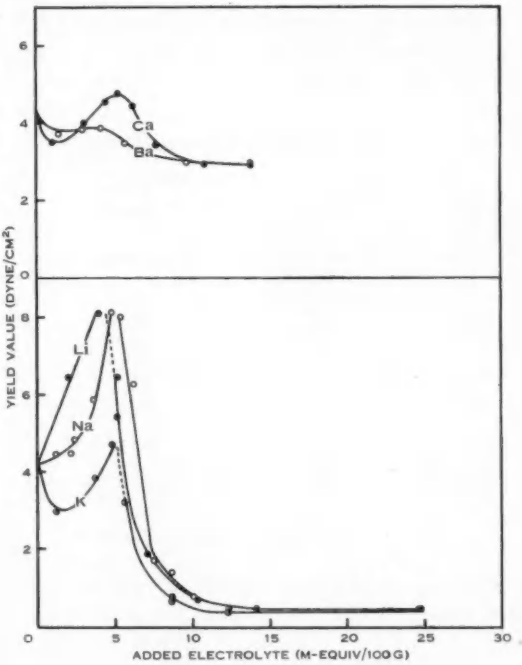

Fig. 2.—Yield value-added electrolyte.

of cup=82·2. The bob was suspended from a steel wire which could be readily changed if desired, however, one such wire covered the range of viscosities encountered in this work. To prevent the bob swinging off-centre whilst in operation, it was supported on a shaft passing through loose centring bearings kept constantly oiled. It was possible to read the deflection scale to $\frac{1}{2}°$. The cup could be slipped readily in and out of a housing, and was mounted in a

container through which water was circulated from a constant temperature bath. The viscometer drive was continuously variable from 0–430 r.p.m. (0–472 sec^{-1}). Actual speed measurement during each reading was achieved by means of a revolution counter and stop-watch.

The torque wire was calibrated with a torsional pendulum after the method of Lillie (1929); it had a wire constant of 737·8 dyne cm/radn.

The immersed end of the bob was corrected for by Green's (1949) method.

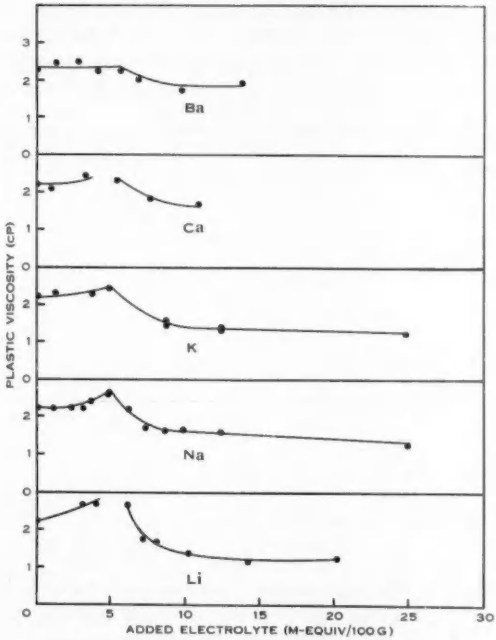

Fig. 3.—Plastic viscosity-added electrolyte.

The two instrument constants (calculated according to Green) were $S=0{\cdot}6819\times10^{-3}$, and $C=0{\cdot}679\times10^{-2}$. Thus,

$$\eta=8{\cdot}39\times\frac{\Delta\theta}{\Delta\text{r.p.m.}}\ \text{(cP)},$$

$$f=0{\cdot}0875\times\theta\ \text{(dyne/cm}^2\text{)}\quad(\theta\text{ in degrees}).$$

The viscosities were measured at 25 °C on 1·03 sp. gr. suspensions of the $17{\cdot}8\times10^4$ cm^2/g fraction of purified Egerton kaolinite prepared as described previously (Street and Buchanan 1956). In all cases a stock slurry of the clay had electrolyte added to it to give the concentration desired, then water to bring the suspension sp. gr. to 1·03. All prepared suspensions were let stand

for 1 week before the viscosity measurements were made. At least six points were recorded at different rates of shear, the plastic viscosity calculated from the slope of the curve, and the yield value from the intercept. The deflection points were tested for equilibrium by moving the scale first slightly above, and then slightly below its apparent equilibrium position and allowing it to regain its original reading.

Measurements carried out (Street 1956) indicate that the axial ratio of these particles is of the order of 11–12, thus the volume concentration necessary to avoid particle interference would be (Goodeve 1939) about 0·14; the value of 0·0194 chosen for this study should be sufficiently below this figure to render the possibility of interference most unlikely.

The results of the runs carried out by neutralizing the H-kaolinite suspension by NaOH, KOH, LiOH, $Ca(OH)_2$, and $Ba(OH)_2$, and calculated as yield value and plastic viscosity are given in Figures 2 and 3 respectively.

Results of runs carried out by the addition of sodium citrate and sodium hydroxide to calcium kaolinite are given in Tables 1 and 2 respectively.

TABLE 1

KAOLIN TREATED WITH SODIUM CITRATE

$Ca(OH)_2$ (m-equiv/100 g)	Sodium Citrate (m-equiv/100 g)	ζ-Potential (mV)	Plastic Viscosity (cP)	Yield Value (dyne/cm²)
7·00	0·00	14·9	1·71	2·99
7·00	0·69	56·3	1·30	1·54
7·00	1·39	81·6	1·17	0·60
7·00	2·08	79·0	1·05	0·17
7·00	2·78	80·7	1·09	0·00

TABLE 2

CA-KAOLIN TREATED WITH SODIUM HYDROXIDE

$Ca(OH)_2$ (m-equiv/100 g)	NaOH (m-equiv/100 g)	ζ-Potential (mV)	Plastic Viscosity (cP)	Yield Value (dyne/cm²)
7·00	0·00	14·9	1·71	2·99
7·61	0·00	17·0	1·65	2·91
7·00	0·62	28·7	1·65	2·91
7·00	1·24	65·0	1·36	2·22
7·00	1·86	69·2	1·36	1·71
7·00	2·48	96·0	1·38	1·28

III. DISCUSSION

Discussion on the effect of ζ-potential on the rheological properties of these suspensions will be confined almost entirely to its effect on the yield value, since it is here that the effect is largely apparent. Figure 4 presents curves of the

apparent viscosity of a Na-kaolinite at various rates of shear, and illustrates clearly why capillary viscometer measurements (i.e. those determined at very high rates of shear) are useless as a diagnostic measure of the interparticulate forces.

If the region from the peak of the yield value curve is considered then it can be seen that there is a very rapid decrease in yield value with rapid increase in ζ-potential (see Fig. 5 taken from Street and Buchanan 1956); that the

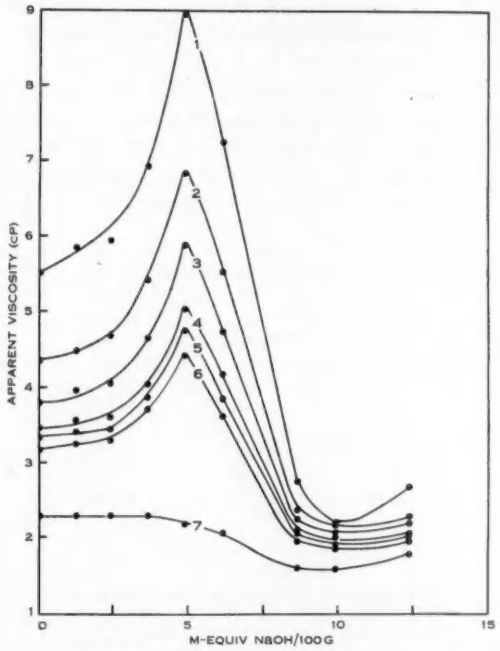

Fig. 4.—Apparent viscosity-added sodium hydroxide.

Curve number:	1	2	3	4	5	6	7
Rate of shear (sec^{-1}):	126	208	285	367	416	472	∞

maximum occurs at the point of inflexion of the ζ-curve; and that the yield value reaches its constant low value at about the same point as the ζ-curve reaches its high, relatively constant, value. In effect, this part of the curve with each base is a measure of the effects of that base when added to a clay already saturated (to the exchange capacity) with the cation of that base.

The yield value relationships in this region can be interpreted by inspection of the potential energy diagrams (Figs. 6 and 7); these have been calculated using the formulae for van der Waal's attraction and for the potential energy of repulsion given by Verwey and Overbeek (1948).

These are

$$V_a = -\frac{A}{48\pi}\left[\frac{1}{d^2} + \frac{1}{(d+\delta)^2} - \frac{2}{(d+\frac{1}{2}\delta)^2}\right], \quad \ldots\ldots\ldots (2)$$

and

$$V_r = \frac{64nkT}{\varkappa} \cdot \gamma^2 \cdot \frac{e^{-2\varkappa d}}{1+e^{-2\varkappa d}}, \quad \ldots\ldots\ldots\ldots\ldots (3)$$

where A = London-van der Waal's constant (2×10^{-12}),
V_a = potential energy of attraction (erg/cm^2),
V_r = potential energy of repulsion (erg/cm^2),
d = half-distance between plates,
δ = thickness of plates,
$n = c\times6\cdot03\times10^{17}$ (c = conc. in m-mol/l),
k = Boltzmann's constant,
T = temperature (°A),
D = dielectric constant,
Z = valence,

$$\varkappa = \left(\frac{8\pi n\varepsilon^2 Z^2}{DkT}\right)^{\frac{1}{2}},$$

$$\gamma = \frac{e^{v/2}-1}{e^{v/2}+1},$$

$$v = \frac{Ze\zeta}{kT}.$$

Since the repulsive potential increases so greatly when the distance of approach is equal to the double layer thickness, that is, when $\varkappa d=1$, it seems possible that the approach of particles in sheared suspensions is never closer than this, thus a plot of V_a+V_r for $\varkappa d=1$ at various electrolyte concentrations should give a clear indication of the point at which the repulsive forces are greatly in excess of the attractive (see also Harmsen, van Schooten, and Overbeek 1953). Figure 7 shows that in fact the electrolyte concentration at which the yield value does suddenly decrease considerably is very close to that concentration at which the repulsive potential shows a marked increase over the attractive. This is true both in Figure 6, where V_a+V_r is plotted for various distances of approach, and in Figure 7, where V_a+V_r is plotted for the specific (and different, according to the electrolyte concentration) distance of $\varkappa d=1$.

It is thus easy to conceive that the yield value in this region is controlled by these forces. When the attractive or flocculating forces are high, then the yield value is high ; on the other hand, when the repulsive forces increase, and there is a greater potential barrier over which the particles must pass before they flocculate, then a lower yield value is exhibited. When the particles are completely deflocculated, there is probably no true yield value in low volume concentration suspensions, although the more concentrated suspensions will probably exhibit one caused by particle interference etc. (Norton, Johnson, and Lawrence 1944).

The early part of the curve shows a behaviour contrary to these ideas, that is, the ζ-potential and the yield value increase simultaneously. It is possible to interpret this behaviour in terms of the hypothesis of negative faces and positive edges on the kaolinite particles. An examination of the graphs shows that the maximum of the yield value always occurs at about 5 m-equiv/100 g of added base, thus at pH's of Li=7·2, Na=7·4, and K=6·8 (Street and Buchanan 1956). It is noteworthy that Schofield and Samson's results show that the change from positive to negative adsorption of Cl^- occurs at about pH 6·5–7·5, and that Johansen's (1955) results indicate that the isoelectric point of the edge faces may be expected at a pH of the order of 6·5.

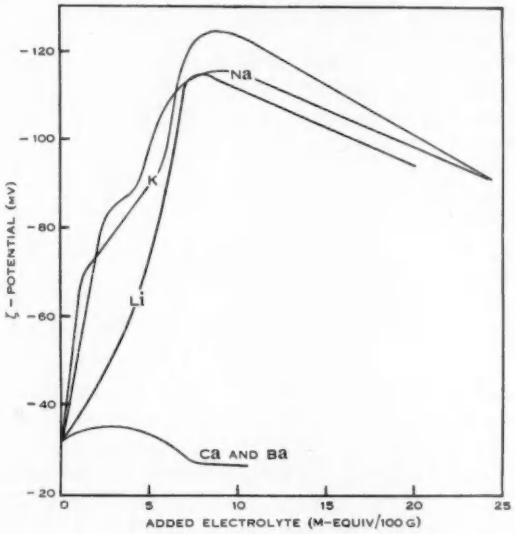

Fig. 5.—ζ-potential-added electrolyte.

The most reasonable interpretation of the behaviour of these suspensions before the equivalence point would appear to be along the lines of that adopted by students of the rheological behaviour of macromolecular colloids (see e.g. Overbeek and de Jong *in* Kruyt 1952). They explain the behaviour of the macromolecule entirely in terms of changes in the expansion equilibrium. The coiled macromolecule exhibits a low apparent viscosity, and the uncoiled or stretched macromolecule exhibits a high apparent viscosity. The coiled-uncoiled equilibrium is determined by the ζ-potential of the units of the polymer, and the hydration of its parts.

In accordance with the ideas of Schofield and Samson, it is believed that edge to face, that is, "bipolar" flocculation exists in the H-kaolinite, and throughout neutralization of the H-clay with base up to some point beyond equivalence. This bipolar flocculation is caused by the attraction of oppositely-

charged areas on the clay surface. It is possible to conceive of the clay floccule in this state as being essentially analogous to a coiled macromolecule; if it should become more tightly coiled its apparent viscosity will decrease, if it should uncoil its apparent viscosity will increase. During shear the " coiled " kaolinite particles will partly stretch and adlineate, and partly (probably) break up into smaller flow units.

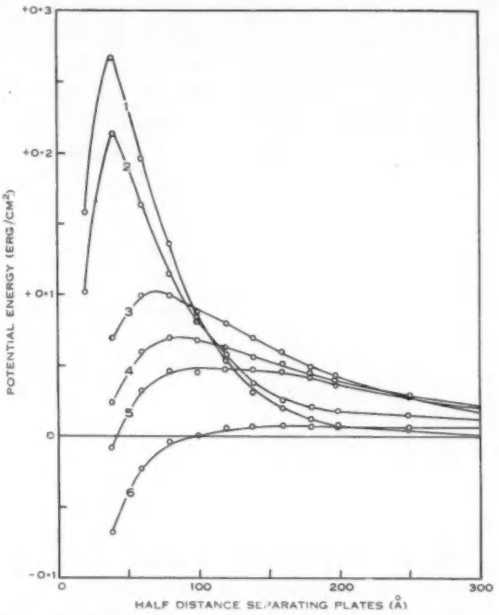

Fig. 6.—Potential energy of interaction ($V_a + V_r$)-half distance of approach of plates.

Curve number:	1	2	3	4	5	6
Added NaOH (m-equiv/100 g):	8·5	8·0	5·25	2·25	1·3	0
ζ-Potential:	124	124	91	76	69	32

There will be two factors that can cause variation in the degree of coiling of the floccule, (*a*) the amount of hydration associated with the particular exchange cation, and (*b*) the repulsive forces arising from the existence of the double-layer potential. In spite of the fact that the floccules can remain linked by bipolar flocculation, the negatively-charged faces will repel each other with a force some function of the magnitude of the ζ-potential, the ionic concentration etc. (eqn. (3)), or with a force proportional to the ionic hydration, and these will cause expansion or contraction of the floccule.

The effect of the ionic hydration may be gauged by either an " expansion index " (U/v), or by the ionic potential (v/r) (Goldschmidt 1937), when U is the

total hydration energy, v is the valency, and r is the radius of the ions concerned. Table 3 gives values of these parameters for the ions considered.* Using either of these parameters to describe the hydration relations of the various cations it would be reasonable to expect that, if hydration were the only factor to be considered, then the gradual neutralization of H-clay would result in tighter coiling of the floccule and lower apparent viscosity for the Na- and K-clays, and uncoiling of the floccule and increased apparent viscosity in the case of the

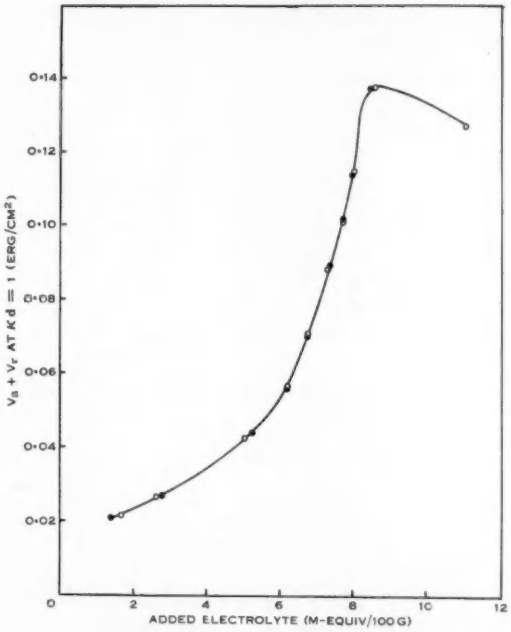

Fig. 7.—Potential energy of interaction (V_a+V_r) at $\kappa d=1$–added electrolyte. ● NaOH; ○ LiOH.

Li-, Ba-, and Ca-clays. Since the effect of the ζ-potential should be, at least up to the point of neutralization of the positive areas, to give higher apparent viscosity with the higher potential of the Li-, Na-, and K-clays, and to give a lower apparent viscosity with the lower potential of the Ba- and Ca-clays, thus it is possible for the resultants of these effects to give the values observed.

(i) *Li-clay.*—The effects of both ζ-potential and expansion index are additive in increasing the apparent viscosity.

* This effect may perhaps be even better described in terms of attraction or repulsion between the cations as effected by the entropies of their co-spheres. This is in turn related to the B-coefficients of their viscosities in aqueous solution. These B-coefficients are: Ca^{2+}, +0·29; Ba^{2+}, +0·15; Li^+, +0·15; H^+, +0·07; Na^+, +0·08; K^+, —0·01.

(ii) *K-clay.*—The effect of the expansion index is to coil the floccules and thus give a lower apparent viscosity, and this at first outweighs the effect of the ζ-potential to uncoil the floccules and give an increased apparent viscosity; later the effect of the high ζ predominates.

(iii) *Na-clay.*—Although the hydration of the Na ion is such that the effect should be to decrease the viscosity, this is outweighed by the effect of the ζ in increasing the viscosity.

For monovalent cations the edges become neutralized at a pH of the order of 7, then at a higher pH they acquire a negative charge with the result that the then high ζ-potential causes the repulsive forces to become predominant, with break up of the floccules, deflocculation, and consequent low yield value. The interaction from this point to further increase in electrolyte concentration follows the previous discussion.

TABLE 3

VALUES FOR IONS CONSIDERED

Ion	Hydration Energy	Expansion Index	Ionic Potential
Ca^{2+}	410	205	1·9
Ba^{2+}	376	188	1·4
Li^{+}	136	136	1·3
H_3O^{+}	130	130	—
Na^{+}	114	114	1·0
K^{+}	94	94	0·75

The clay mineral montmorillonite, with a higher $SiO_2 : Al_2O_3$ ratio, could be expected to exhibit its maximum of viscosity at a lower pH than kaolinite since its edge-face isoelectric point will be lower. This maximum is shown by Garrison and ten Brink (1939), in a study of electrodialysed Wyoming bentonite, to occur at about pH=4·5. Although, they do not explain their results on the hypothesis developed here, nevertheless, they are capable of interpretation along these lines.

The behaviour of the Ca-kaolinite suspension is such as to lend at least circumstantial evidence in support of the general hypothesis. It is to be expected that the edges of the particles will be first neutralized, and then negatively charged at the same pH in the divalent hydroxide solutions as in the monovalent; however, they do not then disperse and exhibit zero yield value. It is consistent with the theory of edge to face flocculation that the increasing hydration associated with the replacement of H^+ by Ca^{++} should cause an increase in viscosity as is actually observed. However, at the pH where the edges are neutralized, there is no sudden increase in ζ-potential, and thus no increase in the repulsive forces between the 001 faces, so that the suspension is not deflocculated, and thus no sudden decrease in yield value occurs. On the other hand, there is a break in the type of flocculation from bipolar to lamellar; the persistence of this lamellar flocculation is the reason for the continued

existence of a considerable yield value. It is a fact that observation under the ultra-microscope reveals that a different size of particle exists in the suspension beyond the equivalence point.

It would be very difficult to interpret the behaviour of the divalent cations except in terms of bipolar flocculation; on the hydration theory the original increase in viscosity is explained by hydration increase, it is difficult however to explain the decrease that occurs at the equivalence point, since the hydration remains high and the ζ low, thus it is not possible to invoke deflocculation. However, the existence of positive edges which are neutralized at this point, and the changeover from one type of flocculation to another gives a very credible explanation of the observed facts. The ζ-potential of monovalent cation clays is reduced at high electrolyte concentration, the attractive forces predominate, and the yield value increases due to the onset of lamellar flocculation.

It is not essential that the pH be increased to 6·5 or greater before deflocculation occurs, provided the positive edges can be neutralized in some other manner. van Wazer and Besmertnuck (1950) showed that polyphosphates are capable of reducing the viscosity without raising the pH. Additions of sodium citrate to this clay when just past saturation with $Ca(OH)_2$ effectively increased the ζ-potential and decreased the yield value.

The importance of the ζ-potential in the deflocculation mechanism is also brought out in the data presented in Table 2 which shows the results of adding NaOH to a clay just past saturation with $Ca(OH)_2$. It is to be noted that filtrates from these clays were proved by analysis to be free of Ca ion, that is, there had been no exchange of Ca by Na.

The curves for plastic viscosity, Figure 3, or the viscosity at infinite shear show, although to a lesser degree, the same pattern as the yield value. It is suggested that the main factor controlling the shape of the plastic viscosity curves is the volume concentration and the shape of the flow units. In the early part of the curve the volume concentration will tend to increase as the flocs immobilize water, then past the equivalence point the deflocculation of the kaolinite will result in the release of the previously immobilized water and decrease of the plastic viscosity. In a similar fashion, the asymmetry of the particles should first increase as the floccules uncoil, and then suddenly decrease, with consequent decrease of the viscosity, on deflocculation.

There will also be an electroviscous effect arising from the charge on the particles; however, it is not possible to calculate this effect with any precision from the data available.

IV. References

Baver, L. D. (1929).—Res. Bull. Mo. Agric. Exp. Sta. No. 129.

Booth, F. (1950).—*Proc. Roy. Soc.* A **203**: 533.

Collie, C. H., Hasted, J. B., and Ritson, D. M. (1948):—*J. Chem. Phys.* **16**: 1.

Garrison, A. D., and ten Brink, K. C. (1939).—*Trans. Amer. Inst. Min. (Metall.) Engrs.* **136**: 175.

Goldschmidt, V. M. (1937).—*J. Chem. Soc.* **1937**: 655.

Goodeve, C. F. (1939).—*Trans. Faraday Soc.* **35**: 342.

GREEN, H. (1949).—" Industrial Rheology and Rheological Structures." (John Wiley & Sons : New York.)

HARMSEN, G. J., VAN SCHOOTEN, J., and OVERBEEK, J. TH. G. (1953).—*J. Colloid Sci.* **8** : 72.

HOLDRIDGE, D. A. (1952).—*Chem. & Ind.* **1952** : 94.

JOHANSEN, P. (1955).—Thesis, University of Melbourne.

JOHNSON, A. L., and NORTON, F. H. (1941).—*J. Amer. Ceram. Soc.* **24** : 189.

KRUYT, H. R. (1952).—" Colloid Science." (Elsevier Publ. Co. Inc. : Amsterdam.)

LILLIE, H. R. (1929).—*J. Amer. Ceram. Soc.* **8** : 505.

MARSHALL, C. E. (1948).—" The Colloid Chemistry of the Silicate Minerals." (Academic Press Inc. : New York.)

NORTON, F. H., JOHNSON, A. L., and LAWRENCE, W. G. (1944).—*J. Amer. Ceram. Soc.* **27** : 149.

VAN OLPHEN, H. (1950).—*J. Inst. Petrol.* **36** : 223.

SCHOFIELD, R. K., and SAMSON, H. R. (1954).—*Disc. Faraday Soc.* **18** : 135.

SMOLUCHOWSKI, M. (1916).—*Kolloidzschr.* **18** : 194.

STREET, N. (1956).—Thesis, University of Melbourne.

STREET, N., and BUCHANAN, A. S. (1956).—*Aust. J. Chem.* **9** : 450.

VERWEY, E. J. W., and OVERBEEK, J. TH. G. (1948).—" Theory of the Stability of Lyophobic Colloids." (Elsevier Publ. Co. Inc. : Amsterdam.)

DE WAELE, A., and LEWIS, G. L. (1953).—*Kolloidzschr.* **133** : 86.

VAN WAZER, J. R., and BESMERTNUCK, E. (1950).—*J. Phys. Chem.* **54** : 89.

STUDIES IN THE CHEMISTRY OF PHENOTHIAZINE

II. DICHLOROPHENOTHIAZINES, AND SOME OBSERVATIONS ON THE HERZ REACTION AND THE SMILES REARRANGEMENT

By K. J. FARRINGTON* and W. K. WARBURTON*

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Summary

The preparation of some dichlorophenothiazines is described. Some limitations of the Herz reaction and of the Smiles rearrangement are discussed. The orientation of some substitution products is established.

I. INTRODUCTION

It was shown in Part I of this series (Farrington and Warburton 1955) that Smiles rearrangement of substituted diphenyl sulphides prepared by treating 2,5-dichloronitrobenzene with chloro-substituted *o*-aminobenzenethiols gave dichlorophenothiazines in high yield and purity. It is now shown that dichlorophenothiazines may be prepared from diphenyl sulphides derived from 2,3-dichloronitrobenzene, but not from 2,4-dichloronitrobenzene and 2,6-dichloronitrobenzene, because ring closure does not occur after Smiles rearrangement of these compounds. With 2-acetamido-3,5-dichlorodiphenyl sulphides, even the rearrangement occurs only in special cases.

The general synthesis of phenothiazines with halogen atoms in any desired position is further limited by the difficulty of preparing 2-amino-3-chlorobenzenethiol (I) and 2-amino-6-chlorobenzenethiol. The Herz reaction cannot be used to prepare *o*-aminobenzenethiols with a free 5-position. 3,7-Dichlorophenothiazine is the only isolable product from the dichlorination of phenothiazine.

II. SUBSTITUTED *o*-AMINOBENZENETHIOLS

When 2,4-dichloronitrobenzene was treated with sodium disulphide in an attempt to find a convenient preparation of 2-amino-5-chlorobenzenethiol, both chlorine atoms suffered some attack, and the product was evidently the polymer represented by the partial structure II. Attempts to replace only the *ortho*-halogen atom by slowly adding the sodium salt to a solution of the nitrobenzene gave nothing recognizable.

2,3-Dichloronitrobenzene reacted with sodium disulphide only on refluxing with a large excess of the reagent, and the product was 2-chloro-6-nitrobenzenethiol, and not the expected disulphide. The nitro-compound was not

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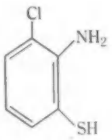
reduced by sodium hydrosulphite, sodium sulphide, sodium disulphide, or ferrous sulphate and ammonia. Reduction with zinc and acetic acid gave the aminothiol only as the insoluble zinc salt, of no preparative value.

The reaction of *p*-chloroaniline with sulphur chloride and hydrolysis of the Herz compound so obtained gives 2-amino-5-chlorobenzenethiol, whereas the same procedure, when applied to *o*-chloroaniline, gives 2-amino-3,5-dichlorobenzenethiol (see Part I). Herz, in the original patent (Cassella and Co. 1922), did not clearly state whether *o*-aminobenzenethiol could be prepared from aniline, but used a symbol for an aromatic ring which might or might not have become substituted. König (1928) deliberately prepared a salt of 2-amino-5-chlorobenzenethiol from aniline by the Herz reaction, but gave no experimental detail of this step. Attempts in this Laboratory to prepare *o*-aminobenzenethiol from aniline have failed, and it is concluded that the Herz reaction is of no value for preparing *o*-aminobenzenethiols with a free 5-position, whether substituted or not.



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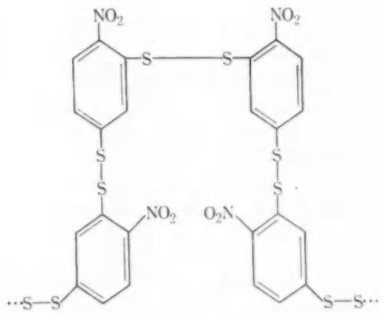

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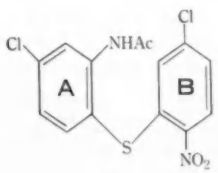

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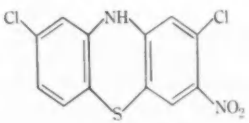

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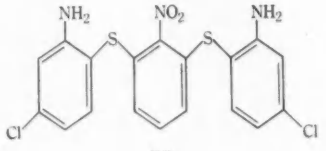
III. The Smiles Rearrangement

Some limitations to the preparation of phenothiazines by the Smiles rearrangement of 2-acetamido-2′-nitrodiphenyl sulphides have been observed. 2-Acetamido-4,5′-dichloro-2′-nitrodiphenyl sulphide (III) failed to give a phenothiazine when heated with sodium hydroxide, sodium ethoxide, or sodium *iso*propoxide in various solvents. However, colour changes usually observed during a Smiles rearrangement took place, and the expected *S*-methyldiphenylamine was isolated in good yield by adding methyl iodide (cf. Evans and Smiles

1935). No dichlorophenothiazine could be isolated after treating 2-acetamido-4,3′-dichloro-2′-nitrodiphenyl sulphide with alkali, but the crude product showed ultraviolet absorption consistent with the presence of a small amount of a dichlorophenothiazine.

It should be noted that it is only when the chlorine atom in ring B is *ortho* or *para* to the nitro-group that it interferes with ring closure. This is therefore probably a resonance effect and not an inductive effect, which causes a fractional negative charge at the carbon atom occupied by the nitro-group (which is probably the position involved in ring closure) and renders it less liable to attack by the negatively charged sulphur atom.

The presence of additional nitro-groups in ring B is known to facilitate ring closure (Kerhmann and Schild 1899). It was found that 2-acetamido-4,5′-dichloro-2′,4′-dinitrodiphenyl sulphide gave 2,8-dichloro-3-nitrophenothiazine (IV), but in such poor yield that elimination of the nitro-group from the phenothiazine was not attempted.



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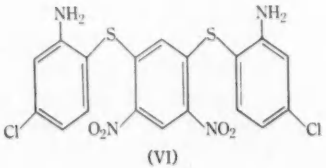

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When ring A contained chlorine atoms in both the 3- and 5-positions, no rearrangement occurred if ring B contained less than three nitro-groups. Reacetylation of the reaction mixture from treatment of 2-acetamido-3,5-dichloro-2′-nitro- and of 2′,4′-dinitrodiphenyl sulphides gave only starting materials. However, the corresponding diphenyl sulphide prepared from picryl chloride rearranged readily, even without acetylation, to give the dark red 1,3-dichloro-7,9-dinitrophenothiazine. 1,3-Dichlorophenothiazine was finally obtained by treating 2,3,5-trichloronitrobenzene with *o*-aminobenzenethiol and rearranging the acetylated diphenyl sulphide. Ring closure occurred readily.

The presence of even traces of dichlorophenothiazines in reaction mixtures was readily detected by ultraviolet absorption, since all these compounds, unless they contain nitro-groups, show strong absorption maxima at about 260 mμ. Most substituted phenothiazines have absorption maxima in the region 253–261 mμ (Cymerman-Craig and Warburton 1956).

IV. Diphenyl Sulphides

2,6-Dichloronitrobenzene, even when in excess, reacts with the sodium salt of 2-amino-4-chlorobenzenethiol to give much of the double replacement product V, as well as the diphenyl sulphide, but the former is less soluble, and remaining traces of its derivative are easily removed after acetylation, which increases the difference in solubility. Similarly, both chlorine atoms of 1,3-dichloro-4,6-dinitrobenzene are highly reactive, and with the sodium salt of 2-amino-4-chlorobenzenethiol, even at −5 °C, only VI is obtained. The diphenyl sulphide may be prepared as the main product by slowly adding alkali to a solution of the nitro-compound and the thiol.

2-Acetamido-4,5′-dichloro-2′-nitrodiphenyl sulphide (III) was made from 2-amino-4-chlorobenzenethiol and 2,4-dichloronitrobenzene. There is no known exception to the rule that nucleophilic compounds react with 2,4-dihalonitrobenzenes preferentially at the 2-position (for references see Bunnett and Morath 1955), and benzenethiol has been found to react normally (Leandri and Tundo 1954).

Recent work by Bunnett and Snipes (1955) has shown, however, that *p*-chloronitrobenzene reacts more rapidly than *o*-chloronitrobenzene with benzenethiol, and confirmation of the structure of III seemed desirable. It was obtained from ultraviolet absorption. The only absorption maximum of III between 230 and 300 mμ is at 244 mμ. The corresponding maxima of 2-acetamido-4,4′-dichloro-2′-nitrodiphenyl sulphide and 2-acetamido-4-chloro-2′-nitrodiphenyl sulphide are at 245 and 239 mμ, respectively, whereas for 2-acetamido-4-chloro-4′-nitrodiphenyl sulphide there is no maximum between 230 and 300 mμ.

V. Orientation of Substituents

The rigorous synthetic methods described above are unsuitable for large-scale preparative work, and this restricts biological tests to small animals. Some more direct methods of obtaining larger amounts of substituted phenothiazines have been examined.

Direct chlorination of phenothiazine gives 3,7-dichlorophenothiazine in very small yield (Unger and Hoffman 1896). The product, m.p. 222 °C, was presumed to have this structure because on nitration and reduction it did not give a positive thionine test. The same authors reported that chlorination by hydrogen chloride in ether, followed by oxidation with oxides of nitrogen, gave a monochlorophenothiazine, a tetrachlorophenothiazine, and 1,3-dichlorophenothiazine. This compound, m.p. 225–227 °C, gave a positive thionine test, and must therefore have a free position *para* to the amino-group.

In this Laboratory, chlorination of phenothiazine with hydrogen chloride after Unger and Hoffman (1896) gave a monochlorophenothiazine, a tetrachlorophenothiazine, and 3,7-dichlorophenothiazine, m.p. 240–241 °C (corr.). This compound was identical in melting point and ultraviolet absorption with that prepared by the Smiles rearrangement. 1,3-Dichlorophenothiazine prepared by the Smiles rearrangement differed from Unger and Hoffman's product in both these properties, and in infra-red absorption. The erroneous conclusion

of Unger and Hoffman is explained by the observation that the melting point of 3,7-dichlorophenothiazine is not depressed by the presence of as much as 10 per cent. of the monochlorophenothiazine which is formed in the same reaction. The latter impurity, which is about 5/7 of the mixture of products soluble in warm ethanol, persists in traces (as shown by chlorine analyses) after as many as 10 fractional crystallizations from various solvents, and even these traces give a positive thionine test. The impurities which lowered the melting point of the product of direct chlorination may have been higher chlorination products, but no analytical figures are reported.

Antonov (1953), in a paper which is available to the authors as an abstract, reported a dichlorophenothiazine of unknown orientation, m.p. 219–220 °C, prepared by treating an ethanolic solution of phenothiazine with hydrogen chloride and then with hydrogen peroxide, and reducing the 5-oxide so formed with lead chloride. This dichlorophenothiazine gave a 10-methyl derivative, m.p. 125 °C, which is probably identical with the 3,7-dichloro-10-methylphenothiazine, m.p. 127 °C, described by Schmalz and Burger (1954).

Attempts to repeat Antonov's work in this Laboratory have been hampered by lack of full experimental detail. Reduction with lead chloride did not succeed, but reduction of the 5-oxide mentioned with stannous chloride gave 3,7-dichlorophenothiazine, identical with that prepared by the Smiles rearrangement. The 3,7-dichlorophenothiazine-5-oxide was accompanied by a product soluble in ethanol, m.p. 174 °C, which gave a negative thionine test, and was shown by synthesis to be 1,3,7-trichlorophenothiazine. It is undoubtedly identical with the trichlorophenothiazine, m.p. 175 °C, prepared by Antonov from the dichlorophenothiazine-5-oxide and hydrogen chloride in ethanol. The reaction of 3,7-dichlorophenothiazine-5-oxide with hydrogen chloride is not unexpected (Page and Smiles 1910; Schmalz and Burger 1954).

It is unlikely that any dichlorophenothiazine except the 3,7-isomer can be prepared from phenothiazine by substitution, and even this isomer, if required in a high state of purity, is best made by the Smiles rearrangement.

VI. Experimental

Melting points are corrected. Analyses are by Dr. K. W. Zimmerman, C.S.I.R.O. Microanalytical Laboratory. Absorption spectra were measured in ethanol, $c = 0{\cdot}5$ or 1 mg/100 ml.

(*a*) *Preparation of Dichloronitrobenzenes.*—2,6-Dichloronitrobenzene was prepared in 5% overall yield from *p*-nitroaniline by the seven-step synthesis of Holleman and Reiding (1904). 2,4-Dichloronitrobenzene was obtained as a by-product from the preparation of the preceding compound by hydrolysis and deamination of 3,5-dichloro-2-nitroacetanilide. The latter compound is the principal product of nitration of 3,5-dichloroacetanilide. Holleman and Reiding (1904) report that it was about 2/3 of the total nitration product. Each of the present authors found that it was about 4/5 of the crude product, the remainder being the *p*-nitroacetanilide. The 2,4-dichloronitrobenzene from this source (which has also been used by Kremers and Bendich 1939) was difficult to purify, and the compound was more conveniently made by nitration (Holleman and Reiding 1904) of *m*-dichlorobenzene (Chattaway and Evans 1896).

(*b*) *Action of Sulphur Chloride on Aniline.*—When aniline was treated with sulphur chloride, and the Herz compound converted through the zinc salt to the sodium salt, exactly as in Part I of this series (Farrington and Warburton 1955) at all stages, the sodium salt of 2-amino-5-chloro-

benzenethiol was obtained in 45–50% overall yield (from aniline). Attempts to avoid nuclear substitution by control of time, temperature, and concentration were unsuccessful. Aniline hydrochloride (Cassella and Co. 1922) gave only the 5-chloro-compound in about the same yield. The sodium salt of the thiol was estimated and identified by neutralization to the free thiol. Further proof of identity was obtained by preparation of 6-chloro-2-phenylbenzothiazole, m.p. 156 °C, undepressed by material, m.p. 156 °C, prepared from *p*-chloroaniline by the Herz reaction (Bogert and Corbitt (1926) report m.p. 156–157 °C) and by condensation of the sodium salt with 2,5-dichloronitrobenzene to give, in about 80% yield, 2-amino-5,4′-dichloro-2′-nitrodiphenyl sulphide (see Part I), m.p. and mixed m.p. 160 °C (decomp.).

(*c*) *2-Chloro-6-nitrobenzenethiol.*—A solution of hydrated sodium sulphide (45 g) and sulphur (5·7 g) in ethanol (180 ml) was cautiously added to a solution of 2,3-dichloronitrobenzene (9·9 g) in ethanol (40 ml), and the dark mixture refluxed for 16 hr under a guard tube. After cooling for 4 hr in the refrigerator, the orange liquid was filtered off, and the residue washed with a little anhydrous ethanol. The dried, almost colourless residue (about 12 g) was dissolved in water (100 ml) and separated from a trace of sulphur, and 10N hydrochloric acid (2 ml) was added with stirring. After 1 hr in the refrigerator the solid was collected, washed twice with ice-cold ethanol (5-ml portions), and dried in a vacuum to give *2-chloro-6-nitrobenzenethiol* as pale fawn, rectangular plates. Solution in sodium bicarbonate, filtration, and reacidification gave almost colourless plates, 4·05 g (42%), m.p. 140–141 °C (decomp.) (Found : C, 38·2, 37·7 ; H, 3·5 3·3 ; O, 16·5 ; N, 7·5 ; S, 16·6 ; Cl, 18·2%. Calc. for $C_6H_4O_2NSCl$: C, 38·0 ; H, 2·1 ; O, 16·9 ; N, 7·4 ; S, 16·9 ; Cl, 18·7%). Steam distillation of the alcoholic filtrate gave a trace of 2,3-dichloronitrobenzene, m.p. 60–64 °C.

(*d*) *Reaction of 2,4-Dichloronitrobenzene with Sodium Disulphide.*—When 2,4-dichloronitrobenzene was refluxed in ethanol with an equivalent amount of sodium disulphide the mixture darkened and sodium chloride separated at once. On cooling, and collecting the organic material, there was obtained an amorphous yellow product which was extracted with boiling acetic acid. The residue softened, but did not melt completely, between 120 and 150 °C, and the material deposited by the acetic acid remained amorphous and no compound of sharp melting point could be obtained from it.

(*e*) *2-Amino-4,6′-dichloro-2′-nitrodiphenyl Sulphide.*—Reaction of 2,3-dichloronitrobenzene (Holleman and de Mooy 1915) with the sodium salt of 2-amino-4-chlorobenzenethiol, as in Part I, Section IV (*c*), gave pale orange *plates*, m.p. 97 °C, in 50% yield (Found : C, 46·1 ; H, 2·7 ; N, 8·4%. Calc. for $C_{12}H_8O_2N_2SCl_2$: C, 45·7 ; H, 2·6 ; N, 8·9%). Acetylation, as in Part I, Section IV (*d*), gave fine lemon-yellow *needles* (from ethanol), m.p. 111 °C, in 60% yield (Found : C, 47·5 ; H, 3·0 ; O, 13·8 ; N, 7·4%. Calc. for $C_{14}H_{10}O_3N_2SCl_2$: C, 47·1 ; H, 2·8 ; O, 13·4 ; N, 7·9%).

(*f*) *1,8-Dichlorophenothiazine.*—Smiles rearrangement of the preceding acetyl compound, as in Part I, Section IV (*e*), gave *1,8-dichlorophenothiazine* as long, violet needles (from ethanol), m.p. 112 °C, in 70% yield (Found : C, 53·7 ; H, 2·8 ; N, 4·9 ; S, 12·1%. Calc. for $C_{12}H_7NSCl_2$: C, 53·7 ; H, 2·6 ; N, 5·2 ; S, 12·0%) ; $\lambda_{max.}$ 261 mμ ; $\log_{10}\varepsilon$ 4·74.

(*g*) *2-Amino-5,6′-dichloro-2′-nitrodiphenyl Sulphide.*—Reaction of the sodium salt of 2-amino-5-chlorobenzenethiol with 2,3-dichloronitrobenzene, as in Section VI (*e*), gave pale brown *needles*, m.p. 99 °C (from ethanol), in 50% yield (Found : C, 46·1 ; H, 2·8 ; N, 8·7%. Calc. for $C_{12}H_8O_2N_2SCl_2$: C, 45·7 ; H, 2·6 ; N, 8·9%). Acetylation, as in (*e*), gave lemon-yellow *needles*, m.p. 167 °C (from ethanol), in 70% yield (Found : C, 47·6 ; H, 2·8 ; N, 7·7%. Calc. for $C_{14}H_{10}O_3N_2SCl_2$: C, 47·1 ; H, 2·8 ; N, 7·9%).

(*h*) *1,7-Dichlorophenothiazine.*—Smiles rearrangement of the preceding acetyl compound, as in Section VI (*f*), gave *1,7-dichlorophenothiazine* as long, pink needles (from ethanol), m.p. 101 °C, in 70% yield (Found : C, 53·9 ; H, 2·9 ; N, 5·0 ; S, 12·1%. Calc. for $C_{12}H_7NSCl_2$: C, 53·7 ; H, 2·6 ; N, 5·2 ; S, 12·0%) ; $\lambda_{max.}$ 261 mμ ; $\log_{10}\varepsilon$ 4·67.

(*i*) *2-Amino-4,5′-dichloro-2′-nitrodiphenyl Sulphide.*—Reaction of the sodium salt of 2-amino-4-chlorobenzenethiol with 2,4-dichloronitrobenzene, as in Section VI (*e*), gave orange *plates* (from ethanol), m.p. 142 °C, in 70% yield (Found : C, 46·1 ; H, 2·7 ; N, 9·0%. Calc. for $C_{12}H_8O_2N_2SCl_2$: C, 45·7 ; H, 2·6 ; N, 8·9%). Acetylation, as in (*e*), gave cream *needles* (from

ethanol), m.p. 174 °C, in 70% yield (Found : C, 47·4 ; H, 2·9%. Calc. for $C_{14}H_{10}O_3N_2SCl_2$: C, 47·1 ; H, 2·8%).

(*j*) *2-Amino-5,5′-dichloro-2′-nitrodiphenyl Sulphide.*—Reaction of the sodium salt of 2-amino-5-chlorobenzenethiol with 2,4-dichloronitrobenzene, as in Section VI (*e*), gave pale yellow *needles* (from ethanol), m.p. 147 °C, in 60% yield (Found : C, 46·0 ; H, 2·8 ; N, 8·7%. Calc. for $C_{12}H_8O_2N_2SCl_2$: C, 45·7 ; H, 2·6 ; N, 8·9%). Acetylation, as in (*e*), gave greenish yellow *plates* (from ethanol), m.p. 154 °C, in 75% yield (Found : C, 47·4 ; H, 3·0 ; O, 13·8 ; N, 7·4%. Calc. for $C_{14}H_{10}O_3N_2SCl_2$: C, 47·1 ; H, 2·8 ; O, 13·4 ; N, 7·9%).

(*k*) *Smiles Rearrangement of 2-Acetamido-4,5′-dichloro-2′-nitrodiphenyl Sulphide.*—The diphenyl sulphide (200 mg) was dissolved in dry acetone (2 ml) and dry ethanol (2 ml), and a solution of sodium hydroxide (28 mg) in ethanol (0·2 ml) added. The mixture was refluxed for ½ hr and cooled, and methyl iodide (200 mg) added. After warming on the water-bath for 20 min the colour faded, and on removal of about 2/3 of the solvent the cooled mixture deposited *N-acetyl-3,3′-dichloro-6-methanethio-6′-nitrodiphenylamine* as pale orange plates (from ethanol), m.p. 150–155 °C (decomp.), 110 mg (55%) (Found : C, 48·9 ; H, 3·4 ; N, 7·7%. Calc. for $C_{15}H_{12}O_3N_2SCl_2$: C, 48·6 ; H, 3·2 ; N, 7·6%).

(*l*) *1,3-Dichloro-7,9-dinitrophenothiazine.*—2-Amino-3,5-dichlorobenzenethiol (470 mg) was warmed with 10N hydrochloric acid (0·24 ml), and picryl chloride (240 mg) in cold ethanol (12 ml) added, followed at once by sodium acetate (40 mg) in water (1 ml). The mixture was vigorously shaken, then boiled for ½ min and allowed to cool. Water (15 ml) was added and the orange solid collected and washed with water and with cold ethanol, then redissolved in ethanol (5 ml). Sodium hydroxide (200 mg) in water (2 ml) was added to the almost boiling solution. An almost black solid separated at once and was recrystallized from glacial acetic acid to give fine, dark red *needles*, m.p. 215 °C (decomp.), 300 mg (35%) (Found : C, 40·9 ; H, 1·7 ; N, 11·9 ; Cl, 19·9%. Calc. for $C_{12}H_5O_4N_3SCl_2$: C, 40·3 ; H, 1·4 ; N, 11·7 ; Cl, 19·8%) ; $\lambda_{max.}$ 243, 298, 460 mμ ; $\log_{1\,0}\varepsilon$ 4·40, 4·29, 3·87. For 1,3-dinitrophenothiazine Kehrmann and Schild (1899) report $\lambda_{max.}$ 238, 296, 465 mμ ; $\log_{10}\varepsilon$ 4·45, 4·25, 3·87.

(*m*) *2-Acetamido-2′,4′-dichloro-6′-nitrodiphenyl Sulphide.*—Reaction of the sodium salt of *o*-aminobenzenethiol with 2,3,5-trichloronitrobenzene (Holleman and van Häften 1921), as in Section VI (*e*), gave *2-amino-2′,4′-dichloro-6′-nitrodiphenyl sulphide* as fine, lemon-yellow needles (from ethanol), m.p. 111–112 °C, in 50% yield. This compound was not obtained quite pure, and was converted as in (*e*) to its *acetyl derivative*, lemon-yellow needles, m.p. 140·5 °C (from acetic acid) (Found : C, 47·4 ; H, 2·7%. Calc. for $C_{14}H_{10}O_3N_2SCl_2$: C, 47·1 ; H, 2·8%).

(*n*) *1,3-Dichlorophenothiazine.*—Smiles rearrangement of the preceding acetyl compound, as in Section VI (*f*), gave *1,3-dichlorophenothiazine* as long, pale fawn needles (from ethanol), m.p. 115 °C, in 50% yield (Found : C, 54·2 ; H, 2·9 ; N, 5·0 ; Cl, 26·7%. Calc. for $C_{12}H_7NSCl_2$: C, 53·7 ; H, 2·6 ; N, 5·2 ; Cl, 26·5%) ; $\lambda_{max.}$ 264 mμ, $\log_{10}\varepsilon$ 4·70.

(*o*) *2-Amino-3,5-dichloro-2′-nitrodiphenyl Sulphide.*—Reaction of the sodium salt of 2-amino-3,5-dichlorobenzenethiol with *o*-chloronitrobenzene gave long, pale green *needles* (from ethanol), m.p. 204 °C, in 50% yield (Found : C, 46·2 ; H, 2·7 ; N, 8·8%. Calc. for $C_{12}H_8O_2N_2SCl_2$: C, 45·7 ; H, 2·6 ; N, 8·9%). This compound was acetylated by dissolving it in acetic anhydride (3 ml/g) and adding acetyl chloride (1 ml/g) to the cold solution. Small, yellow *needles*, m.p. 174 °C, crystallized from the reaction mixture (Found : C, 47·5 ; H, 2·9 ; N, 7·5%. Calc. for $C_{14}H_{10}O_3N_2SCl_2$: C, 47·1 ; H, 2·8 ; N, 7·9%).

(*p*) *2-Amino-3,5-dichloro-2′,4′-dinitrophenyl Sulphide.*—Reaction of the sodium salt of 2-amino-3,5-dichlorobenzenethiol, as in Section VI (*e*), with 1-chloro-2,4-dinitrobenzene gave fine, yellow *needles* (from acetic acid), m.p. 173 °C, in 55% yield (Found : C, 40·6 ; H, 2·2 ; N, 11·8%. Calc. for $C_{12}H_7O_4N_3SCl_2$: C, 40·0 ; H, 1·9 ; N, 11·6%). Acetylation as in Section VI (*o*), followed by addition of ice and water, gave lemon-yellow *plates* (from acetic acid), m.p. 236 °C (Found : C, 42·6 ; H, 2·6 ; N, 10·3%. Calc. for $C_{14}H_9O_5N_3SCl_2$: C, 41·9 ; H, 2·2 ; N, 10·5%).

(*q*) *Reaction of 1,3-Dichloro-4,6-dinitrobenzene with 2-Amino-4-chlorobenzenethiol.*—Addition of an aqueous solution of the sodium salt of the thiol to an equivalent quantity of the nitro-

compound (Zincke 1909) in acetone at —5 °C, followed by shaking for 1 hr, gave an almost quantitative yield (based on the amount of nitro-compound not recoverable by steam distillation) of *4,6-bis-(2-amino-4-chlorobenzenethio)-1,3-dinitrobenzene*, magnificient orange cubes (from acetic acid), m.p. 264 °C (decomp.) (Found : C, 45·3 ; H, 2·6 ; N, 11·4 ; Cl, 15·3%. Calc. for $C_{18}H_{12}O_4N_4S_2Cl_2$: C, 44·8 ; H, 2·5 ; N, 11·6 ; Cl, 14·7%). Addition, during 1 hr, of the theoretical amount of 10% ethanolic sodium hydroxide at 50 °C to a stirred mixture of equivalent quantities of the thiol and the nitro-compound in ethanol, followed by addition of water, gave a 60% yield of *2-amino-4,5'-dichloro-2',4'-dinitrodiphenyl sulphide* as yellow needles (from ethanol), m.p. 165 °C (Found : N, 11·4%), or as cream needles (from ethanol), m.p. 108–109 °C (Found : N, 11·2%. Calc. for $C_{12}H_7O_4N_3SCl_2$: N, 11·6%). Acetylation of either form of this compound in acetic anhydride containing a trace of sulphuric acid gave cream *needles* (from acetic acid), m.p. 225 °C (Found : C, 42·4 ; H, 2·3%. Calc. for $C_{14}H_9O_5N_3SCl_2$: C, 41·9 ; H, 2·2%).

(*r*) *2,8-Dichloro-3-nitrophenothiazine.*—Smiles rearrangement of the preceding acetyl compounds as in Section VI (*f*) gave pale red *needles* (from ethanol), m.p. 199 °C (decomp.), in 5% yield (Found : N, 9·0%. Calc. for $C_{12}H_6O_2N_2SCl_2$: N, 9·0%).

(*s*) *Reaction of 2,6-Dichloronitrobenzene with 2-Amino-4-chlorobenzenethiol.*—The sodium salt of the thiol (2·10 g) was added to a solution of the nitrobenzene (2·45 g) in ethanol (30 ml) and the mixture refluxed for 4 hr and steam distilled to give 2,6-dichloronitrobenzene, m.p. 67–69 °C (0·47 g). The residue was slowly crystallized from ethanol (30 ml) to give orange crystals, 0·45 g. Two recrystallizations from ethanol gave *2,6-bis-(2-amino-4-chlorobenzenethio)-nitrobenzene*, 0·35 g, orange needles, m.p. 194 °C (Found : C, 49·8 ; H, 2·8 ; N, 9·3 ; S, 15·0%. Calc. for $C_{18}H_{13}O_2N_3S_2Cl_2$: C, 49·3 ; H, 3·0 ; N, 9·6 ; S, 14·6%). Concentration of the filtrate under reduced pressure to 10 ml gave *2-amino-4,3'-dichloro-2'-nitrodiphenyl sulphide*, yellow plates, m.p. 144 °C, 0·40 g (Found : C, 45·4 ; H, 2·9 ; N, 9·0%. Calc. for $C_{12}H_8O_2N_2SCl_2$: C, 45·7 ; H, 2·6 ; N, 8·9%). Acetylation of the last-mentioned compound in acetic anhydride containing a trace of sulphuric acid gave a 70% yield of pale fawn *needles* (from ethanol), m.p. 149 °C (Found : N, 7·3%. Calc. for $C_{14}H_{10}O_3N_2SCl_2$: N, 7·9%).

(*t*) *2-Acetamido-4-chloro-2'-nitrodiphenyl Sulphide.*—Reaction of the sodium salt of 2-amino-4-chlorobenzenethiol and *o*-chloronitrobenzene, followed by acetylation as in Section VI (*e*) gave lemon-yellow *needles* (from ethanol), m.p. 183 °C (Found : C, 52·4 ; H, 3·3 ; N, 8·7%. Calc. for $C_{14}H_{11}O_3N_2SCl$: C, 52·6 ; H, 3·5 ; N, 8·8%).

(*u*) *2-Acetamido-4-chloro-4'-nitrodiphenyl Sulphide.*—Reaction of the sodium salt of 2-amino-4-chlorobenzenethiol and *p*-chloronitrobenzene, followed by acetylation, as in Section VI (*e*), gave lemon-yellow *needles* (from ethanol), m.p. 168 °C (Found : C, 52·3 ; H, 3·3 ; N, 8·8%. Calc. for $C_{14}H_{11}O_3N_2SCl$: C, 52·6 ; H, 3·5 ; N, 8·8%).

(*v*) *2-Acetamido-5,4',6'-trichloro-2'-nitrodiphenyl Sulphide.*—Reaction of the sodium salt of 2-amino-5-chlorobenzenethiol and 2,3,5-trichloronitrobenzene, followed by acetylation as in Section VI (*e*), gave short brown *needles* (from ethanol), m.p. 202 °C (Found : C, 43·2 ; H, 2·4 ; N, 6·9%. Calc. for $C_{14}H_9O_3N_2SCl_3$: C, 42·9 ; H, 2·3 ; N, 7·2%).

(*w*) *1,3,7-Trichlorophenothiazine.*—Smiles rearrangement of the preceding compound as in Section VI (*f*) gave a pale brown product which sublimed at 120 °C/0·01 mm as colourless *needles*, m.p. 176 °C (Found : C, 47·3 ; H, 2·2 ; N, 4·7%. Calc. for $C_{12}H_6NSCl_3$: C, 47·5 ; H, 2·0 ; N, 4·6%) ; $\lambda_{max.}$ 264·5 mμ.

(*x*) *Action of Hydrogen Chloride and Hydrogen Peroxide on Phenothiazine.*—Phenothiazine (20 g) in 96% ethanol (300 ml) was treated at 40–50 °C with hydrogen chloride (45 g) in ethanol (300 ml), and 30% hydrogen peroxide (33 ml) was added with stirring at 40–46 °C during 70 min. The mixture was cooled to room temperature and the solid extracted with ethanol leaving a residue (13·8 g) of 3,7-dichlorophenothiazine-5-oxide, m.p. 232 °C. Reduction of the oxide (3·0 g) with stannous chloride (1·9 g) in ethanol saturated with hydrogen chloride at 50 °C for 5 hr gave, after several recrystallizations and vacuum sublimations, 3,7-dichlorophenothiazine, 0·7 g, m.p. 241–242 °C (Found : C, 54·4 ; H, 2·6 ; N, 4·7 ; Cl, 26·6%. Calc. for $C_{12}H_7NSCl_2$: C, 53·7 ; H, 2·6 ; N, 5·2 ; Cl, 26·5%). The melting point was not depressed by material prepared by the Smiles rearrangement, which, after many recrystallizations and two vacuum sublimations had m.p. 241–242 °C.

The ethanolic extract from the original reaction gave 1,3,7-trichlorophenothiazine (3·2 g) which, after sublimation, had m.p. 174 °C, not depressed by the material described in Section VI (*w*) (Found: C, 47·9; H, 2·0; N, 4·2; Cl, 34·5%. Calc. for $C_{12}H_6NSCl_3$: C, 47·5; H, 2·0; N, 4·6; Cl, 35·1%).

VII. Acknowledgment

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VIII. References

Antonov, D. S. (1953).—*Bull. Inst. Chim. Acad. Bulgare Sci.* **2**: 75. (*Chem. Abstr.* **49**: 6266.)
Bogert, M. T., and Corbitt, H. B. (1926).—*J. Amer. Chem. Soc.* **48**: 783.
Bunnett, J. F., and Morath, R. J. (1955).—*J. Amer. Chem. Soc.* **77**: 5051.
Bunnett, J. F., and Snipes, R. F. (1955).—*J. Amer. Chem. Soc.* **77**: 5422.
Cassella and Co. (1922).—Ger. Pat. 360,690 (Oct. 6). (*Fortschr. Teerfarb.* **14**: 908 (1926).)
Chattaway, F., and Evans, R. (1896).—*J. Chem. Soc.* **69**: 848.
Cymerman-Craig, J., and Warburton, W. K. (1956).—*Aust. J. Chem.* **9**: 294.
Evans, W. J., and Smiles, S. (1935).—*J. Chem. Soc.* **1935**: 1263.
Farrington, K. J., and Warburton, W. K. (1955).—*Aust. J. Chem.* **8**: 545.
Holleman, A. F., and van Häften, F. E. (1921).—*Rec. Trav. Chim. Pays-Bas* **40**: 67.
Holleman, A. F., and de Mooy, W. J. (1915).—*Rev. Trav. Chim. Pays-Bas* **35**: 1.
Holleman, A. F., and Reiding, M. J. (1904).—*Rec. Trav. Chim. Pays-Bas* **23**: 357.
Kehrmann, F., and Schild, L. (1899).—*Ber. dtsch. chem. Ges.* **32**: 2606.
König, W. (1928).—*Ber. dtsch. chem. Ges.* **61**: 2065.
Kremers, C., and Bendich, A. (1939).—*J. Amer. Chem. Soc.* **61**: 2660.
Leandri, G., and Tundo, A. (1954).—*Ann. Chim. Appl. Roma* **44**: 271. (*Chem. Abstr.* **49**: 15783.)
Page, H. J., and Smiles, S. (1910).—*J. Chem. Soc.* **97**: 1115.
Schmalz, A. C., and Burger, A. (1954).—*J. Amer. Chem. Soc.* **76**: 5455.
Unger, O., and Hoffman, K. (1896).—*Ber. dtsch. chem. Ges.* **29**: 1362.
Zincke, T. (1909).—*Liebigs Ann.* **370**: 302.

THE STRUCTURE OF CRYPTOPLEURINE AND HOFMANN DEGRADATION OF SOME QUINOLIZIDINE ALKALOIDS

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Summary

With the help of (—)-cryptopleurine-(^{14}C)-methiodide it has been shown that during attempted Hofmann degradation no structural change takes place. Therefore, the structure of the alkaloid cryptopleurine is I. The formation of racemic quaternary methiodides under conditions which usually effect Hofmann degradation is discussed.

I. INTRODUCTION

The 2′,3′,6′-trimethoxyphenanthro(9′,10′-2,3)quinolizidine structure has been deduced for the racemic *iso*cryptopleurine methiodide from the analysis of X-ray diffraction data (Fridrichsons and Mathieson 1954, 1955). Though the structure of cryptopleurine itself does not necessarily follow from this identification, the same authors suggested that cryptopleurine might be the parent compound I of the methiodide. The work to be described establishes that (—)-cryptopleurine methiodide does constitute the laevorotatory component of the racemic *iso*cryptopleurine methiodide, and therefore the correctness of the above suggestion. Attempts to resolve the quaternary racemate with the aid of *d*-tartaric and *d*-camphor-10-sulphonic acids were unsuccessful and a procedure involving the use of a radioactive tracer was devised. If (—)-cryptopleurine-(^{14}C)-methiodide, prepared from the alkaloid and (^{14}C)-methyl iodide, were recrystallized in the presence of (±)-cryptopleurine methiodide with no labelled atom, the ^{14}C-labelled molecules would appear in crystals of both the laevorotatory and racemic modifications and after recrystallization the concentration of the labelled molecules in the (—)-component of the racemate and in the laevorotatory cryptopleurine methiodide would be the same. Thus if such uniformity of distribution is observed when "*iso*cryptopleurine methiodide" is crystallized in the presence of (—)-cryptopleurine-(^{14}C)-methiodide, then and not otherwise "*iso*cryptopleurine methiodide" must be identical with racemic (±)-cryptopleurine methiodide, and cryptopleurine itself must have the phenanthroquinolizidine structure I. By dissolving one part of (—)-cryptopleurine-(^{14}C)-methiodide together with four parts of the unlabelled racemate in methanol about two-thirds of the original ^{14}C-labelled molecules should appear in the recrystallized racemate and, as seen in Section II (*b*) (iv), the calculated and experimentally-determined values are in excellent agreement. Such deviation as was observed is ascribed to slight decomposition of the methiodide during recrystallization from water. The possibility that random ionization of the

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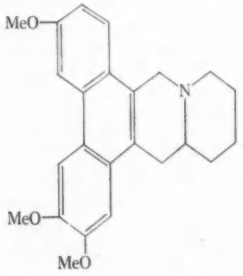
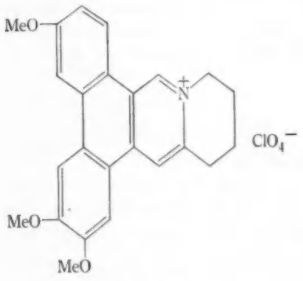
methyl group might be responsible for the observed radioactivity of the racemate was conclusively eliminated, as the amount of radioactivity remained unchanged after refluxing the ^{14}C-labelled racemate with a very large excess of unlabelled methyl iodide. Since structural change should not occur either in the course of methiodide formation or during recrystallization the structure of cryptopleurine is established as I. The name *iso*cryptopleurine should therefore be replaced by (±)-cryptopleurine.

The racemization of (−)-cryptopleurine methiodide under conditions which might be expected to lead to Hofmann degradation, and which do not affect cryptopleurine itself, calls for discussion of this reaction, and it has been postulated (Gellert 1955) that the racemization is due to opening and



(I)



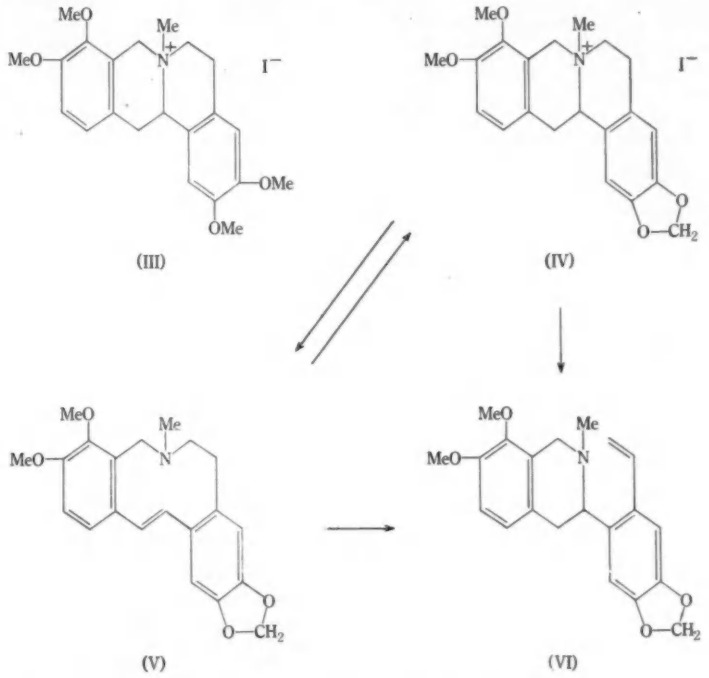
(II)

reclosing of the quinolizidine ring between the nitrogen and the bridgehead carbon atoms.* This explanation is supported, as reported previously, by the behaviour of (−)-tetrahydropalmatine and (−)-canadine methiodides. Both α- and β-(−)-tetrahydropalmatine methiodides yield the same racemic methiodide (III) in nearly quantitative yield when refluxed with 20 per cent. aqueous potassium hydroxide for 2½ hr, only traces of a methine base being detected. On the other hand, (−)-canadine-α-methiodide gives racemic canadine methiodide but in addition undergoes Hofmann degradation to a considerable extent. Three compounds have been isolated from the action of alkali on (−)-canadine-α-methiodide, the racemic canadine methiodide, an iodide which can be converted to the methiodide, and a base isolated as its hydrochloride, which is identical with a base B obtained by Pyman (1913) by Hofmann degradation under conditions differing from those applied here. Pyman isolated three bases for which formulae V and VI were assigned, and showed that V could be converted to the racemic form of IV and, through the methohydroxide of IV, to the racemic base B (VI).

Through the courtesy of Professor A. Albert and Mr. T. M. Sharp we obtained from the Wellcome Laboratories of Tropical Medicine samples of Pyman's bases A, B, and C (the optically active isomer of B) for measurement of their

* Racemization in the quinolizidinium system has also been considered by Lewis and Shoppee (1956).

ultraviolet absorption spectra. These spectra (Fig. 1) support the conclusion arrived at above, namely, that the base isolated as its hydrochloride is identical with Pyman's base B (VI), and that the third product is a modification of canadine methiodide. The spectra also reveal that the base A, which on Pyman's evidence undoubtedly possessed the structure V allocated to it originally, has been reconverted on standing to a quaternary canadine methosalt. This is borne out both by the m.p. and solubility of the material, neither of which is in accordance with Pyman's original data. This transformation confirms



(III) (IV) (V) (VI)

the greater stability of two fused 6-membered rings compared with the 10-membered ring, and supports the hypothesis that racemization of the bridgehead carbon atom can occur through an intermediate V. The racemization of (−)-cryptopleurine methiodide can then be explained by an intermediate analogous to V. Such an explanation is preferable to one not involving cleavage of the C-N bond at the bridgehead, particularly as neither (−)-cryptopleurine nor (−)-canadine racemize on treatment with alkali. Epimerization at the bridgehead carbon atom of the quinolizidine portion of the molecule in the yohimbine and reserpine series (i.e. at C_3) is brought about by refluxing the alkaloids with alkali in ethylene glycol for 6 hr (MacPhillamy *et al.* 1955). But in these alkaloids there are other active centres in the molecule and in any event

both (−)-cryptopleurine and (−)-canadine are decomposed under the conditions used. Such reaction as does occur when the refluxing time is reduced does not involve racemization; the product from cryptopleurine is optically active ($[\alpha]_D$ −105°) and possibly arises by demethylation.

It was reported previously (Gellert and Riggs 1954) that racemic cryptopleurine methiodide yields cryptopleurine when heated with glycollic potassium hydroxide at 150 °C for 2 hr. However, this was based only on the m.p. and mixed m.p. of the base and its methiodide, the rotation was not measured.

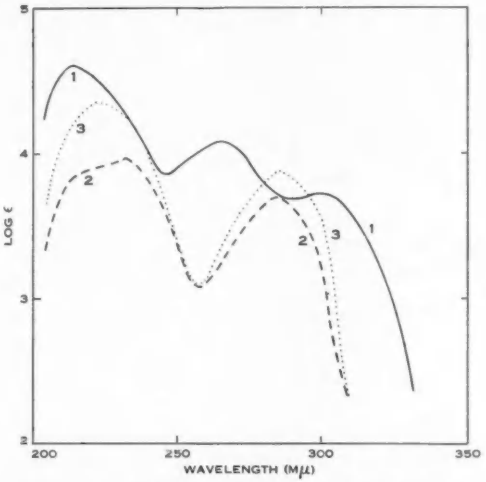


Fig. 1.—Ultraviolet absorption in ethanolic solution (measured with a Hilger Uvispek).

Curve 1: Pyman's base B; Pyman's base C (? hydrochloride); hydrochloride of base, m.p. 252–253 °C, from Hofmann degradation of (—)-canadine-α-methiodide.

Curve 2: (—)-Canadine-α-methiodide; Pyman's (—)-canadine-α-methochloride; Pyman's base A (?).

Curve 3: Methiodide, m.p. 160–162 °C, from Hofmann degradation of (—)-canadine-α-methiodide.

Because of the difficulty of reconciling the formation of cryptopleurine in this way with the conclusion that "*iso*cryptopleurine" is (±)-cryptopleurine, the experiment has been repeated. The product is, in fact, optically inactive and its methiodide melts at 264–268 °C. However, it does not consist entirely of (±)-cryptopleurine suggesting that some demethylation of the methoxyl group may take place with glycollic potassium hydroxide in glycol even at 150 °C.

The sensitivity of (−)-cryptopleurine towards light, air, and solvents (Gellert and Riggs 1954) is due to oxidation as a tetradehydro-derivative (isolated as its perchlorate) was formed when the base was allowed to stand in chloroform

for 2 weeks unprotected from light. Structure II is suggested for this product. (—)-Cryptopleurine reacts with cyanogen bromide giving a bromocyanamide derivative which gave only intractable products on treatment with a variety of reagents.

II. Experimental

All melting points are corrected unless otherwise stated. Analyses were carried out by the C.S.I.R.O. Microanalytical Laboratory, under the direction of Dr. K. W. Zimmermann.

(*a*) *Resolution Experiments.*—(i) (±)-Cryptopleurine methochloride (1·5 g) was dissolved in water and stirred in the cold with freshly precipitated silver hydroxide. After filtering from silver salts 1 g of *d*-camphor-10-sulphonic acid was added and the solution evaporated to dryness. The residue was crystallized several times from a 1 : 4 mixture of ethanol-ethyl acetate giving colourless needles of (±)-*cryptopleurine metho*-d-*camphor-10-sulphonate*, m.p. 137–139 °C, $[\alpha]_D^{18}$ +12±1° (*c*, 1·0 in MeOH) (Found : C, 62·4 ; H, 7·6 ; O, 23·0 ; N, 1·9 ; S, 4·7 ; loss of wt. at 56 °C/12 mm, 6·7%. Calc. for $C_{35}H_{45}O_7NS.2{\cdot}5H_2O$: C, 62·8 ; H, 7·5 ; O, 22·7 ; N, 2·1 ; S, 4·8 ; $2{\cdot}5H_2O$, 6·7%). The crystals and the mother liquors were each converted to the quaternary iodide. In both instances the racemic methiodide was isolated, having m.p. and mixed m.p. 270–272 °C.

(ii) Attempted resolution with *d*-tartaric acid was carried out as described above. Both the crystals and the mother liquors yielded the same racemate when converted to the iodide.

(iii) Direct conversion of (±)-cryptopleurine methiodide to the methohydroxide with silver hydroxide in hot methanol was accompanied by oxidation, precipitation of iodine and, to some extent, Hofmann degradation giving *cryptopleurine methine*, m.p. 190–192 °C, after recrystallization from benzene (Found : C, 77·1 ; H, 7·5 ; O, 12·7 ; N, 3·7%. Calc. for $C_{25}H_{29}O_2N$: C, 76·7 ; H, 7·5 ; O, 12·3 ; N, 3·6%). The mixed m.p. with (—)-cryptopleurine is 174–176 °C.

Table 1

Activity Measurements*

Compound	Total Count	Time of Count (min)	Counts/Min
A	106800	20	5340
B	69364	26	2668
C	9259	20	463
D	9208	20	460·5
E	9083	20	454
F	12098	25	484
G	9322	20	466

* Measurements carried out by Dr. K. R. Lynn. All activity measurements were made under the conditions described above. Background count was constant.

(*b*) *Radioactive Tracer Experiments.*—(i) *Preparation of* (—)-*Cryptopleurine*-(^{14}C)-*methiodide.* To (—)-cryptopleurine (150 mg) covered with 15 ml of benzene *c.* 600 mg of methyl iodide containing $^{14}CH_3I$ (about 0·02 mc activity) was added. The mixture was sealed in a tube and warmed in a water-bath for 2½ hr. The gelatinous product was crystallized several times from methanol until the m.p. remained constant at 215–217 °C, and the substance exhibited a constant count of radioactivity (*A* Table 1), measured as an " infinitely thick " sample of (—)-cryptopleurine-(^{14}C)-methiodide on 2 cm^2 planchet under an end-window (mica) Geiger counter. The homogeneity of the compound was established by mixing it with an equal weight of unlabelled (—)-cryptopleurine methiodide and recrystallizing the mixture from methanol, when the count was halved (*B* Table 1) under the same condition as before.

(ii) *Preparation of* (±)-*Cryptopleurine-*(^{14}C)-*methiodide.* Unlabelled (±)-cyrptopleurine methiodide (200 mg) and labelled (—)-cryptopleurine methiodide (*B*, 50 mg) were dissolved in methanol (50 ml), the solution slowly concentrated to about 20–25 ml, and allowed to stand overnight. The crystals which separated (220 mg, *C* Table 1) and which must contain some (—)-cryptopleurine methiodide, were recrystallized from methanol giving *D* (Table 1), and the recrystallization repeated giving *E* (Table 1). Finally, because of the desirability of crystallizing from more than one solvent system (Calvin *et al.* 1949) *E* was recrystallized from water giving sample *F* (Table 1). The counts recorded for each of these specimens are presented in Table 1. The somewhat higher count observed with *F* is probably due to the recrystallization from water causing some loss of iodine.

(iii) *Exclusion of the Possibility of Random Ionization.* (±)-Cryptopleurine-(^{14}C)-methiodide (110 mg) was dissolved in methanol (30 ml), unlabelled methyl iodide (2 ml) added, and the mixture refluxed for 2½ hr. The solution was then concentrated to about 10 ml and after addition of a few drops of unlabelled methyl iodide, allowed to crystallize. The product was recrystallized from methanol in the presence of a few drops of unlabelled methyl iodide giving sample *G* (Table 1).

(iv) *Calculation of Activity.* If (—)-$C_{24}H_{27}O_3N.^{14}CH_3I.2H_2O$ (50 mg) (corresponding to 48·38 mg (—)-$C_{24}H_{27}O_3N.^{14}CH_3I.H_2O$) is mixed with (—)-$C_{24}H_{27}O_3N.CH_3I.H_2O$ (100 mg) and (+)-$C_{24}H_{27}O_3N.CH_3I.H_2O$ (100 mg) (i.e. 200 mg of (±)-$C_{24}H_{27}O_3N.CH_3I.H_2O$) the racemate obtainable from the mixture should contain 32·67 mg (—)-$C_{24}H_{27}O_3N.^{14}CH_3I.H_2O$ representing 67·52% of the original activity. If the specific activity of the original methiodide (*X*) equals a/50 counts min^{-1} m-equiv^{-1} then the specific activity of the racemate separating after admixture (*Y*) should be $67{\cdot}52/10^2 \times a/200$ counts min^{-1} m-equiv^{-1} and the ratio

$$\frac{Y}{X}=\frac{50}{a}\cdot\frac{67{\cdot}52\times a}{20\times 10^2}=16{\cdot}9\%.$$

Count on the starting material:	2668	counts min^{-1}
16·9% of the starting count:	451	,, ,,
Count on *E* (Table 1):	454	,, ,,
Count on *G* (Table 1):	466	,, ,,

(c) *Tetradehydrocryptopleurine Perchlorate.*—A solution of (—)-cryptopleurine in chloroform deposited yellow crystals in substantial amounts after standing for 2 weeks without protection from light. The material was converted into the perchlorate which after recrystallization from methanol-acetone had m.p. 298–300 °C (decomp.) after darkening above 280 °C (uncorr.) (Found: C, 59·5; H, 5·1; O, 24·3; N, 3·1; Cl, 7·5; loss of wt. at 80 °C/12 mm, 2·5%. Calc. for $C_{24}H_{25}O_3N.HClO_4.0{\cdot}5H_2O$: C, 59·7; H, 5·2; O, 24·8; N, 2·9; Cl, 7·3; $0{\cdot}5H_2O$, 1·9%). The base is evidently quaternary since after basification nothing was extractable with benzene as is the case with cryptopleurine.

(d) *von Braun Degradation of* (—)-*Cryptopleurine.*—A solution of (—)-cryptopleurine and cyanogen bromide in acetone was refluxed for 2 hr. The product which separated on cooling was repeatedly crystallized from methanol. *Cryptopleurine bromocyanamide* was obtained in colourless needles, m.p. 171–172 °C (Found: C, 62·0; H, 5·6; N, 5·5; Br, 16·4%. Calc. for $C_{25}H_{27}O_3N_2Br$: C, 62·1; H, 5·6; N, 5·8; Br, 16·5%).

(e) *Reaction of* (—)-*Cryptopleurine with Glycollic Potassium Hydroxide.*—(i) (—)-Cryptopleurine was refluxed with 10% glycollic potassium hydroxide for 6 hr, then the mixture was diluted with an equal volume of water, and extracted with chloroform. The very small amount of *base* remaining after evaporation of the chloroform extract was converted to its *hydrochloride*, m.p. 265–267 °C, which was depressed to 260–263 °C on mixing with authentic (—)-cryptopleurine hydrochloride.

(ii) (—)-Cryptopleurine refluxed with 10% glycollic potassium hydroxide for 2 hr, and worked up as above, yielded a *base* with an indefinite m.p., with $[\alpha]_D^{20}$ —105° (*c*, 0·4 in $CHCl_3$). Its hydrochloride melts at 265–267 °C and its *methiodide* at 240–245 °C.

(iii) (+)-Cryptopleurine methiodide was heated in 10% glycollic potassium hydroxide at 150 °C for 2 hr. The isolated base, m.p. 192–196 °C (uncorr.), was (±)-cryptopleurine, $[\alpha]_D^{30} = \pm 0°$ (*c*, 1·0 in chloroform). The base formed a methiodide, m.p. 264–268 °C, which gave a weak ferric chloride reaction and was obviously impure (±)-cryptopleurine methiodide.

(*f*) (—)-*Tetrahydropalmatine Methiodides.*—A solution of (—)-tetrahydropalmatine in acetone containing methyl iodide on standing deposited crystals of (—)-*tetrahydropalmatine-α-methiodide*, which after recrystallization from methanol-acetone had m.p. 248–251 °C, $[\alpha]_D^{21}$ —118° (*c*, 0·41 in MeOH) (Found : C, 53·5 ; H, 6·1 ; O, 12·9 ; N, 2·5 ; I, 25·6 ; (N)-Me, 2·8%. Calc. for $C_{22}H_{28}O_4NI$: C, 53·1 ; H, 5·7 ; O, 12·9 ; N, 2·8 ; I, 25·5 ; Me, 3·0%).

Addition of acetone to the mother liquors after the separation of the α-methiodide precipitated (—)-*tetrahydropalmatine-β-methiodide*, which after recrystallization from acetone containing a little methanol had m.p. 210–213 °C, $[\alpha]_D^{21}$ —125° (*c*, 0·55 in MeOH) (Found : C, 52·9 ; H, 5·8 ; O, 13·5 ; N, 2·9 ; I, 24·9 ; (N)-Me, 2·7%. Calc. for $C_{22}H_{28}O_4NI$: C, 53·1 ; H, 5·7 ; O, 12·9 ; N, 2·8 ; I, 25·5 ; Me, 3·0%).

(*g*) *Attempted Hofmann Degradation of Tetrahydropalmatine Methiodides.*—(i) When (—)-tetrahydropalmatine-α-methiodide was refluxed with 20% aqueous potassium hydroxide for 2½ hr the (±)-*tetrahydropalmatine methiodide* separated in almost quantitative yield. The crystals were washed with ether and the aqueous alkaline mother liquor was extracted with ether yielding only a trace of a methine base, which could not be purified. The (±)-methiodide crystallized from methanol as colourless needles, m.p. 251–254 °C, $[\alpha]_D^{21}$ ±0° (*c*, 0·4 in MeOH) (Found : C, 53·4 ; H, 5·9 ; O, 13·1 ; N, 2·8 ; I, 25·1 ; (N)-Me, 2·8%. Calc. for $C_{22}H_{28}O_4NI$: C, 53·1 ; H, 5·7 ; O, 12·9 ; N, 2·8 ; I, 25·5 ; Me, 3·0%).

(ii) Under the same reaction conditions tetrahydropalmatine-β-methiodide also produced only a trace of a methine base and the same (±)-tetrahydropalmatine methiodide in almost quantitative yield, m.p. 251–254 °C, $[\alpha]_D^{21}$ ±0° (*c*, 0·19 in MeOH). The m.p. of this (±)-methiodide was not depressed on admixture with the sample obtained from the α-methiodide.

(*h*) *Hofmann Degradation of* (—)-*Canadine-α-methiodide.*—(i) (—)-Canadine-α-methiodide (from *Xanthoxylum veneficum* F. M. Bail.) was refluxed with 20% aqueous potassium hydroxide for 2 hr. The solid which precipitated was still laevorotatory. It was therefore again refluxed with 20% aqueous potassium hydroxide after which the process was again repeated. The final product was washed with ether and the combined aqueous alkaline liquors extracted with ether. Evaporation of the combined ethereal solutions left a non-crystallizable base which was converted to a *hydrochloride*, m.p. 252–253 °C, after crystallization from methanol (Found : C, 64·9 ; H, 6·3 ; N, 3·5 ; MeO, 15·6 ; (N)-Me, 3·0%. Calc. for $C_{21}H_{23}O_4N.HCl$: C, 64·7 ; H, 6·2 ; N, 3·6 ; 2MeO, 15·9 ; Me, 3·8%). Pyman reports m.p. 258 °C for his base B hydrochloride.

The aqueous alkaline liquors, after extraction with ether, were extracted with chloroform giving an *iodide* R_F 0·69 in BuOH-AcOH which after recrystallization from methanol-acetone melted at 160–162 °C, resolidified above 180 °C, and then remelted at 235–243 °C (decomp.) (Found : C, 51·6 ; H, 5·7 ; O, 15·1 ; N, 2·6 ; I, 24·3 ; MeO, 14·2%. Calc. for $C_{21}H_{24}O_4NI.0·5H_2O$: C, 51·4 ; H, 5·1 ; O, 14·7 ; N, 2·8 ; I, 25·9 ; 2MeO, 12·6%). A mixture with (±)-canadine methiodide (see below) gave no depression in m.p. (235–243 °C), and shows only partial melting at 160 °C. The product which separated initially from the aqueous alkaline solution gave, after recrystallization from methanol, (±)-*canadine methiodide*, m.p. 243–245 °C (after darkening above 210 °C), $[\alpha]_D^{21}$ ±0° (*c*, 0·2 in MeOH), R_F 0·48 in BuOH-AcOH (Found : C, 51·5 ; H, 5·3 ; O, 14·8 ; N, 2·7 ; I, 24·8 ; MeO, 12·3 ; (N)-Me, 2·8 ; loss of wt., 0·7%. Calc. for $C_{21}H_{24}O_4NI.0·5H_2O$: C, 51·4 ; H, 5·1 ; O, 14·7 ; N, 2·8 ; I, 25·9 ; 2MeO, 12·6 ; Me, 3·0 ; $0·5H_2O$, 1·8%).

(ii) The specimen of Pyman's base A melted at 141–154 °C (uncorr.) and was insoluble in ethyl acetate. Pyman records m.p. 135–136 °C for the base which he recrystallized from ethyl acetate. The specimen of Pyman's base B melted at 114–115 °C (uncorr.), lit. 114–115 °C. The specimen of Pyman's base C melted at 195–215 °C (uncorr.) after sintering above 150 °C ;

it was evidently mainly the hydrochloride as it dissolves in hot water and the aqueous solution gives a white precipitate with silver nitrate solution. Pyman records m.p. 101–102 °C for the base and 229 °C for the hydrochloride.

III. Acknowledgments

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IV. References

Calvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M., and Yankwich, P. E. (1949).—"Isotopic Carbon." p. 241. (John Wiley & Sons: New York.)

Fridrichsons, J., and Mathieson, A. McL. (1954).—*Nature* **173**: 132.

Fridrichsons, J., and Mathieson, A. McL. (1955).—*Acta Cryst.* **8**: 761.

Gellert, E. (1955).—*Chem. & Ind.* **1955**: 983.

Gellert, E., and Riggs, N. V. (1954).—*Aust. J. Chem.* **7**: 113.

Lewis, H. R., and Shoppee, C. W. (1956).—*J. Chem. Soc.* **1956**: 313.

MacPhillamy, H. B., Huebner, C. F., Schlittler, E., St. André, A. F., and Ulshafer, P. R. (1955).—*J. Amer. Chem. Soc.* **77**: 4335.

Pyman, F. L. (1913).—*J. Chem. Soc.* **103**: 817.

THE CARDIAC GLYCOSIDES OF *GOMPHOCARPUSF RUTICOSUS* R.BR.

I. AFROSIDE

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Summary

Afroside, $C_{29}H_{42}O_9$, is a new cardiac glycoside which has been obtained from *Gomphocarpus fruticosus* R.Br. It contains a carbonyl (aldehyde) group at C_{10}, and a secondary hydroxyl group in the nucleus, which has been placed provisionally at position C_{11}. On the basis of the available evidence, afroside appears to consist of a mixture of the isomeric free aldehyde and the 19–11 cyclic hemiacetal forms. Of these substances, the cyclic hemiacetal form is the only one which has been isolated in pure condition (afroside B). Acetylation of afroside produced a triacetate, $C_{35}H_{46}O_{12}.H_2O$, which is identical with that obtained by the acetylation of afroside B. Reduction of afroside with sodium borohydride produces afrosidol, $C_{29}H_{44}O_9$, which shows no evidence for a carbonyl group at C_{10}. Hydrolysis of afroside produces α-anhydroafrogenin, $C_{23}H_{28-30}O_5.H_2O$, which forms a monoacetate, $C_{25}H_{32}O_7.H_2O$. The infra-red spectra of these compounds show the presence of a saturated γ-lactone ring in the structure of the nucleus, besides the normal $\Delta^{\alpha-\beta}$-γ-lactone ring at C_{17} (1786, 1755, 1633 cm^{-1}). Acetyl-α-anhydroafrogenin also shows an intense absorption band of simple structure at 1238 cm^{-1}, which indicates an equatorially orientated acetyl group at C_3. As all the naturally occurring cardiac aglycones of known structure have a β-orientated hydroxyl group at C_3, for the substituent in this position to be equatorial, the A/B ring junction is probably *trans*.

Hydrolysis of afrosidol produces α-anhydroafrogenol, $C_{23}H_{32}O_5.H_2O$, which forms a diacetate, $C_{27}H_{36}O_7.H_2O$. The infra-red spectrum of α-anhydroafrogenol shows no evidence of the saturated γ-lactone ring which is present in the structure of α-anhydroafrogenin.

I. INTRODUCTION

Gomphocarpus fruticosus R.Br., variously known as *Asclepias fruticosa* L., "milk-weed", and "narrow leaved cotton bush", for some time has been known to be toxic to animals (Watt and Breyer-Brandwijk 1932; Hurst 1942; Webb 1948). When samples of this plant, obtained from Queensland, were tested for the presence of cardioactive constituents, they gave positive indication of the presence of these substances (Thorp and Watson 1953).

Reichstein and co-workers (Keller and Reichstein 1949; Hunger and Reichstein 1952*a*, 1952*b*) have isolated the two cardiac glycosides gofruside and frugoside from the seeds of *G. fruticosus* grown in South Africa. Comparative paper chromatography on formamide-impregnated paper has shown that these glycosides are different from the glycosides isolated from this plant grown in Australia (Watson and Wright 1954). These two substances which have been isolated have been named afroside and gomphoside.

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The mixture of crude glycosides was obtained from the dried whole plant by continuous extraction, firstly with light petroleum and then with 50 per cent. chloroform-methanol. The chloroform-methanol extract, after evaporation and dilution with water, was shaken with carbon tetrachloride to remove pigments. The aqueous alcoholic extract was then evaporated to a small volume, from which the glycosides slowly crystallized (0·084 per cent. of the dry weight of the plant).

A second batch of plant material was defatted with light petroleum and then extracted with 80 per cent. ethanol-water. The aqueous ethanolic extract, after evaporation and dilution with water, was treated with basic lead acetate, filtered, and extracted with chloroform. The yield of mixed glycosides from this extraction was 0·074 per cent. It was found that the lead precipitate contained a small amount of the cardiac glycosides, which could be recovered by dissolving the precipitate in dilute sulphuric acid and re-extracting with chloroform. As this difficulty was not encountered in the first method of extraction, it was used for all subsequent extractions.

The component glycosides of this mixture were separated by chromatography on neutral alumina. The glycosides were applied to the column in a mixture of chloroform-benzene (1 : 1) and eluted by varying the polarity of the eluting solvent (Table 1). The material obtained from fractions 5–10 has been named gomphoside (the chemistry of which will be discussed in a later paper), and that from fractions 16–23, afroside. Paper chromatography, using formamide-impregnated paper, showed gomphoside to be a pure substance, but afroside to consist of two components (I and II). The average yield of crystalline afroside was 0·07 per cent. and that of crystalline gomphoside 0·01 per cent. of the dry weight of the plant material.

Although it has not been demonstrated conclusively that the two constituents of afroside exist in equilibrium, some reactions suggest that these substances may be readily changed from one to the other.

Fresh ethanolic solutions of the mixture have the typical ultraviolet absorption maximum of the butenolide side chain at 217 mμ ($\log \varepsilon = 4 \cdot 22$), and a second maximum at 292·5 mμ ($\log \varepsilon = 1 \cdot 57$; Fig. 1). After the solutions have been stored at room temperature for 4 weeks, this second maximum is replaced by a point of inflexion at that wavelength.

Treatment of afroside (I and II) with hydrochloric acid in acetone (Mannich hydrolysis procedure, Mannich and Siewert 1942) resulted in the recovery of an homogeneous substance in 80 per cent. yield, which was shown by comparative paper chromatography to be identical with one of the constituents of afroside. This compound II, which was named afroside B, analysed for the formula $C_{29}H_{42}O_9$, and gave a positive reaction for the presence of carbohydrate (Bally, Mohr, and Reichstein 1951). The ultraviolet absorption spectrum of afroside B was similar to that of the stored afroside solution.

An attempt to separate the two constituents of afroside by chromatography of the acetates on alumina resulted in a yield of approximately 85 per cent. of an homogeneous acetylated product, 14 per cent. of unacetylated afroside, and a

trace of a third apparently acetylated compound. The main fraction, the acetylated compound III, analysed for the formula $C_{35}H_{46}O_{12}.H_2O$. An acetyl determination indicated the presence of three acetyl groups.

Acetylation of the pure compound, afroside B (II), gave a substance which was shown by analysis, physical constants, and paper chromatography to be identical with the acetate III obtained from the acetylation of afroside. This compound, acetylafroside B, on saponification with potassium bicarbonate in aqueous methanol, gave afroside B. A benzoate could not be obtained in crystalline form.

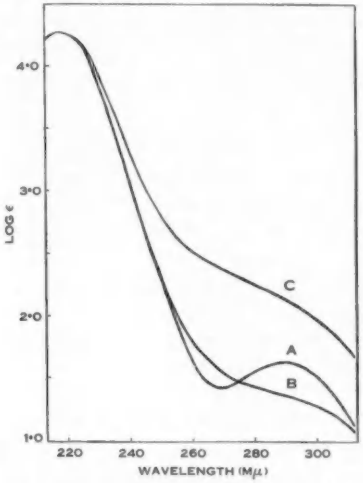


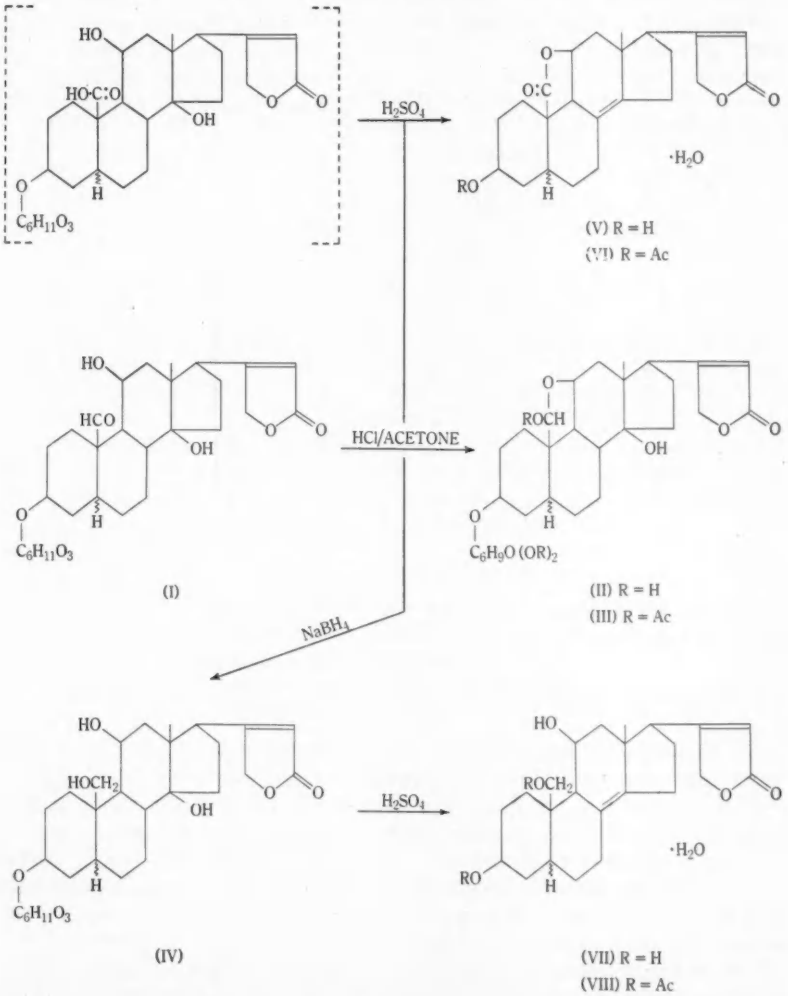
Fig. 1.—Ultraviolet absorption spectra in alcohol. *A*, Afroside, fresh solution. *B*, Afroside, stored solution. *C*, Afrosidol.

Reduction of afroside (I and II) with sodium borohydride gave a chromatographically homogeneous compound which analysed for the formula $C_{29}H_{44}O_9$ (IV) and which had an ultraviolet absorption spectrum similar to that of afroside B (max. 218 mμ, log ε=4·22 ; inflexion 288 mμ, log ε=1·75 ; Fig. 1), except the intensity of absorption in the region 265–325 mμ was somewhat greater than that of afroside B. Chromatography of the reduced compound, afrosidol (IV), on formamide-impregnated paper showed it to be different from both of the constituents of afroside.

An attempt to form an oxime of one of the constituents of afroside produced a mixture of two substances, one of which was identified as afroside B by paper chromatography, but the other could be neither crystallized nor identified positively as an oxime.

Afroside could not be hydrolysed by either the Mannich method or by refluxing with 0·1N sulphuric acid. Hydrolysis under more drastic conditions,

5 per cent. sulphuric acid in methanol-water (75 : 25), gave an homogeneous crystalline product in nearly theoretical yield. This compound, by hydrogenation experiments and infra-red spectroscopy was shown to be α-anhydro-



afrogenin (V). It gave positive Legal, Raymond, and tetranitromethane reactions and analysed for the formula $C_{23}H_{28-30}O_5.H_2O$. Acetylation of α-anhydroafrogenin gave a crystalline monoacetate, $C_{25}H_{32}O_7.H_2O$ (VI) which was unstable to chromium trioxide in acetic acid, but was not oxidized by the

chromium trioxide-pyridine complex used by Poos *et al.* (1953), for the selective oxidation of hydroxyl groups in the presence of double bonds. This reaction indicates that there are no free primary or secondary hydroxyl groups in the structure of this compound.

Hydrolysis of afrosidol (IV), under the same conditions as used for afroside, gave α-anhydroafrogenol (VII). This compound analysed for the formula $C_{23}H_{32}O_5.H_2O$, and gave positive Legal, Raymond, and tetranitromethane reactions. It was shown to be different from α-anhydroafrogenin by paper chromatography. Acetylation of α-anhydroafrogenol gave an homogeneous compound VIII, which could not be crystallized even after chromatography on alumina, but which analysed for the formula $C_{27}H_{36}O_7.H_2O$. An acetyl determination on this compound indicated the presence of two acetyl groups.

The reactions which have been carried out on afroside may be interpreted in the formulae on p. 500.

II. Discussion

As all the above compounds give positive Raymond and Legal reactions, and have the typical ultraviolet absorption maximum of the butenolide ring between 216–218 mμ (log ε approx. 4·2), the presence of this side chain is assumed in each case.

The two anhydrogenins obtained by the acid hydrolysis of afroside and afrosidol were shown to be the α-isomers by hydrogenation experiments and infra-red spectral analyses similar to those described by Cardwell and Smith (1954) in their work on the α- and β-anhydrodigitoxigenins and -digoxigenins. The nuclear double bond (positive tetranitromethane reaction) in both compounds could not be reduced by hydrogenation in neutral solution, but was readily hydrogenated in acid solution using platinum oxide as catalyst.

The infra-red spectrum of α-anhydroafrogenin (V) showed a very weak absorption band in the 12 μ region, but no apparent band in the 6 μ region, which would indicate that there was a small quantity of the $\Delta^{14,15}$ compound present as an impurity in the sample.

The optical rotation of α-anhydroafrogenin (V) is, $[\alpha]_D^{17}$ +60·8°, and that of α-anhydroafrogenol (VII) is $[\alpha]_D^{20}$ +45·8°, which according to Cardwell and Smith (loc. cit.) would indicate that these two compounds are the α-isomers.

The infra-red absorption spectra of α-anhydroafrogenin and acetyl-α-anhydroafrogenin are shown in Figure 2. In both spectra, apart from the normal butenolide absorption bands at 1633 cm^{-1} (6·12 μ), C=C stretching frequency, and 1755 cm^{-1} (5·70 μ), C=O stretching frequency, there is an intense absorption band at 1786 cm^{-1} (5·60 μ). The infra-red spectra of all naturally occurring cardiac glycosides and aglycones have an absorption band of very weak intensity at this wavelength which Jones and Herling (1954) ascribe to the C=O stretching of the butenolide carbonyl group. However, the C=O stretching frequency of the carbonyl group of a saturated γ-lactone, as in the dihydrocardiac aglycones, occurs between 1786 and 1770 cm^{-1} (5·60–5·65 μ), and has a high intensity. Consequently, the intense absorption band at this

wavelength in the spectra of α-anhydroafrogenin and its acetate, must be due to a saturated γ-lactone ring present in the structure of these compounds.

The possibility of the 1786 cm^{-1} band being due to the presence of small quantities of the corresponding butanolide has been excluded by chromatography on the formamide-impregnated system (Zaffaroni, Burton, and Keutman 1949, 1950, 1951 ; Schindler and Reichstein 1951), the system of Svensen and Jensen (1950), and of Silberman and Thorp (1953), and locating the spots with trichloroacetic acid which will detect butanolides. Also, the intensity of the 1786 cm^{-1} band is such that a large amount of the butanolide would have to be present as an impurity.

The infra-red absorption spectrum of α-anhydroafrogenol (VII) (Fig. 2) shows only a very weak absorption band at 1786 cm^{-1} (5·60 μ), as well as the two characteristic bands of the butenolide side chain at 1755 cm^{-1} (5·70 μ),

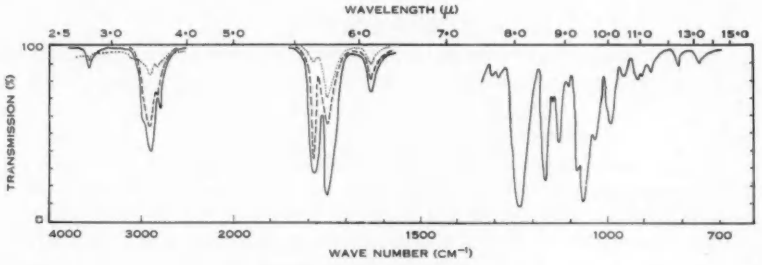


Fig. 2.—Infra-red absorption spectra.

···· α-Anhydroafrogenol : saturated solution in $CHCl_3$, NaCl prism.

---- α-Anhydroafrogenin : 2·5–4 μ CS_2 solution, CaF_2 prism ; 5·5–6·5 μ CCl_4 solution, NaCl prism.

—— Acetyl-α anhydroafrogenin : 2·5–6·5 μ CS_2 solution, CaF_2 prism ; 7–14 μ CCl_4 solution, NaCl prism.

and 1633 cm^{-1} (6·12 μ). The absence of the strong absorption band at 1786 cm^{-1} indicates that the saturated γ-lactone ring is not present in the structure of α-anhydroafrogenol.

The presence of a saturated γ-lactone in the structure of an α-anhydrocardiac genin may be accommodated in only a limited number of positions. Assuming that the aldehyde group is situated at C_{10}, the hydroxyl group in the γ-position with respect to the aldehyde group may be at C_2, C_4, C_6, or C_{11}. Of these structures, those which have the hydroxyl group at C_2 and C_4 necessitate the presence of an α-glycol grouping in the structure of α-anhydroafrogenol. As α-anhydroafrogenol is not oxidized by periodic acid, these two structures can be eliminated. By analogy with cardiac genins of known constitution the structure in which the β-hydroxyl group is at C_{11} is favoured, as there are no known cardiac genins which have a hydroxyl group at C_6.

The formation of the γ-lactone ring of α-anhydroafrogenin is possible by the oxidation of the aldehyde group at C_{10} to the corresponding acid. As the

acid hydrolysis of the glycoside would tend to increase the rate of oxidation of the aldehyde group, it would also facilitate the formation of a lactone of the carboxylic acid with the γ-hydroxy group. Auto-oxidation of the C_{10} aldehyde has been shown to occur in the case of gofruside (Hunger and Reichstein 1952*b*) and also the squill type of glycoside, bovoside A (Katz 1953).

As mentioned previously, α-anhydroafrogenin on acetylation formed the monoacetate, acetyl-α-anhydroafrogenin (VI). This compound was unstable to chromium trioxide in acetic acid, but insufficient oxidation product was available for analyses or constant determinations. However, the reduction of the chromium trioxide in this case is not direct evidence for the presence of an unacetylated hydroxyl group as the $\Delta^{8,14}$ bond would undergo allylic oxidation with the formation of the 15-, or 7-oxo-8(14)-enyl oxide (Wintersteiner and Moore 1943). Acetyl-α-anhydroafrogenin was stable to the chromium trioxide in pyridine reagent described by Poos *et al.* (1953) for the oxidation of primary and secondary hydroxyl groups. This reagent has the advantage over the chromium trioxide-acetic acid reagent in that it does not oxidize double bonds. Thus the stability of acetyl-α-anhydroafrogenin to chromium trioxide in pyridine would indicate that there are no free primary or secondary hydroxyl groups in this molecule.

Acetyl-α-anhydroafrogenol (VIII) was not resistant to oxidation by chromium trioxide in acetic acid and an attempted oxidation of this compound by the chromium trioxide-pyridine complex gave only a few milligrams of a neutral fraction which could not be identified. Whilst not conclusive evidence, this reaction would tend to support the presence of an unesterified hydroxyl group in the structure of this compound.

As α-anhydroafrogenol forms only a diacetate, the third hydroxyl group in the molecule must be resistant to acetylation by acetic anhydride in pyridine. The 11β-hydroxyl group in steroids is not acetylated under these conditions whereas the 11α-hydroxyl group is readily esterified.

On the basis of these experiments it is possible to draw some conclusions regarding the possible structure of the constituents of afroside. It is postulated that afroside consists of a mixture of the two isomeric glycosides I and II, but it has not been demonstrated that these compounds exist in equilibrium. The ultraviolet absorption spectrum of the fresh solution of afroside shows a typical carbonyl absorption band at 292·5 mμ (log ε=1·57), which is replaced on storage of the solution, by a point of inflexion at this wavelength. The change in the nature of the chromophoric group in this case may be explained by the transformation of the free aldehyde form I to the more stable cyclic hemiacetal form II, rather than oxidation to the acid, as stored solutions of afroside do not show acidic reactions.

When afroside is treated with hydrochloric acid in acetone, the sole product is afroside B (II) which has an ultraviolet absorption spectrum consistent with that of an hydroxyl functional group at C_{19} rather than a carbonyl group. Hunger and Reichstein (1952*a*, 1952*b*) consider that corotoxigenin in solution exists in equilibrium between the free aldehyde form and the 19–3 cyclic hemiacetal form, and similarly, aldosterone (Schindler and Reichstein 1954) exists

as an equilibrium between the C_{18} aldehyde and the 18–11 cyclic hemiacetal forms.

The acetylation of afroside results in the formation of an homogeneous triacetate (acetylafroside B (III)), which also shows no evidence of carbonyl absorption in the ultraviolet spectrum. The hydroxyl group at C_{19} in afroside B, would acetylate under the conditions of acetylation (acetic anhydride in pyridine at room temperature).

The reduction of afroside with sodium borohydride produces only afrosidol (IV). As it is unlikely that the cyclic hemiacetal ring of afroside B (II) would be reduced directly to a primary alcoholic group, it appears that this reaction must proceed by the conversion of afroside B to the free aldehyde form I, which then undergoes the reduction to afrosidol (IV). (The yield of afrosidol in these experiments was approximately 70 per cent.)

In the infra-red spectrum of acetyl-α-anhydroafrogenin, an intense absorption band, due to the C—O stretching vibration of the ester carbonyl group, occurs at 1238 cm^{-1} (8·08 μ). This band has the simple structure associated with equatorially orientated substituents at C_3 (Jones *et al.* 1951 ; Cole 1952 ; Cole, Jones, and Dobriner 1952 ; Fürst *et al.* 1952 ; Klyne 1954). As all the naturally occurring cardiac aglycones of known structure have a β-orientated hydroxyl group at C_3, for the substituent in this position to be equatorial, the A/B ring junction is probably *trans*. Thus for α-anhydroafrogenin, its precursors and derivatives, the A/B ring junction is likely to be *trans*.

III. Experimental

Paper Chromatography.—Formamide-Impregnated Paper Systems. Chromatograms on formamide-impregnated paper (Zaffaroni, Burton, and Keutmann 1949, 1950, 1951 ; Schindler and Reichstein 1951 ; Heftman and Levant 1952 ; Jensen 1953) were carried out at 33 °C by the horizontal method (Meredith and Sammons 1952). The solvent systems used with the formamide-impregnated paper were :

(i) Chloroform-benzene (9 : 1), saturated with formamide.

(ii) Chloroform-benzene-*n*-butanol (78 : 12 : 5), saturated with formamide.

Whatman No. 1 paper was used for all chromatograms on the formamide-impregnated paper.

Reversed Phase Systems. The reversed phase technique of Tschesche, Grimmer, and Seehofer (1953) was used with the solvent systems :

(i) *n*-Octanol-*n*-pentanol-water-formamide (6 : 2 : 8 : 2).

(ii) *n*-Octanol-*n*-pentanol-water-formamide (6 : 2 : 1 : 4).

Whatman No. 4 paper was used for all chromatograms on the reversed phase systems.

The developed spots on both types of chromatogram were located by application of the Raymond reaction (Raymond 1938, 1939).

Physical Measurements. Melting points are uncorrected. Ultraviolet absorption spectra were determined in ethanol on a Unicam S.P. 500 ultraviolet spectrophotometer, and infra-red spectra on Perkin-Elmer double-beam instruments by Dr. A. R. H. Cole, University of Western Australia, and by Messrs. Timbrol Ltd., Sydney. Optical rotation measurements were determined in a 1 dm micropolarimeter tube. Analyses were carried out by Dr. K. W. Zimmerman, C.S.I.R.O. Microanalytical Laboratory.

Extraction of the Plant Material.—Dried powdered plant material (3·64 kg) was defatted with light petroleum in a Soxhlet apparatus, and then extracted with 50% chloroform-methanol solution for 20 hr. The chloroform-methanol extract (10 l.) was evaporated to 1·5 l., diluted to

2 l. with water, and then shaken out with 3×250 ml portions of carbon tetrachloride, which removed appreciable quantities of chlorophyll and other pigment material. Extraction of the aqueous-methanolic extract with chloroform (4×250 ml) brought the glycosides into the chloroform solution. Evaporation of the chloroform under reduced pressure left a tarry residue which was redissolved in methanol, diluted with water, and allowed to stand. From this solution 3·03 g of impure crystals of the mixed glycosides were obtained (0·084%).

During the chloroform extraction of the aqueous methanolic solution, 6·8 g (equivalent to 1·87%) of rutin separated at the interface. This pigment was identified by paper chromatography and colour reactions (cf. Casteel and Wender 1953) of the glycoside and the derived aglycone (quercetin), and analyses and physical constants of these compounds.

A second extraction of the plant material was conducted using the dried plant (2·5 kg). This sample was defatted with light petroleum and then extracted with 80% ethanol-water in a Soxhlet apparatus for 26 hr. The ethanolic extract (10 l.) was evaporated under reduced pressure to 1·5 l. and then diluted to 3·0 l. with water. An aqueous solution of basic lead acetate was then added until no further precipitation occurred. The solution was filtered and lead acetate in excess removed by precipitation with disodium phosphate solution (10%). After filtering, the extract was evaporated under reduced pressure to 2 l., and then extracted with chloroform in a continuous liquid-liquid extractor. Evaporation of the chloroform left a tarry residue which was redissolved in methanol, diluted with water, and crystallized. The yield of mixed glycosides from this extraction was 1·86 g, which represents 0·074% of the plant material. The total yield of crude glycosides obtained from five extractions of plant material was 10·34 g.

TABLE 1

SEPARATION OF AFROSIDE AND GOMPHOSIDE ON ALUMINA

Fraction Number	Solvent*	Weight of Residue (mg)	Raymond Reaction
1–3	$CHCl_3 : C_6H_6$ (1 · 1)	14	—ve
4	$CHCl_3 : C_6H_6$ (2 · 1)	6	+ve
5	$CHCl_3 : C_6H_6$ (3 : 1)	16	+ve
6–8	$CHCl_3$	188	+ve
9–11	$CHCl_3 : C_2H_5.O.C_2H_5$ (4 : 1)	58	+ve
12–14	$CHCl_3 : C_2H_5.O.C_2H_5$ (1 : 1)	4	—ve
15–18	$CHCl_3 : C_2H_5.O.C_2H_5$ (1 : 1)	67	+ve
19–20	$CHCl_3 : CH_3COCH_3$ (9 : 1)	148	+ve
21	$CHCl_3 : CH_3COCH_3$ (4 : 1)	63	+ve
22	$CHCl_3$: MeOH (99 : 1)	17	+ve
23	$CHCl_3$: MeOH (98 : 2)	10	+ve
24–27	$CHCl_3$: MeOH (95 : 5 to 1 : 1)	3	+ve

* $CHCl_3$, chloroform : C_6H_6, benzene ; $C_2H_5.O.C_2H_5$, ether ; CH_3COCH_3, acetone.

Separation of the Cardiac Glycosides on Alumina.—The mixed glycosides (1·2 g) were dissolved in dry chloroform (200 ml) and diluted to 400 ml with benzene. This solution was applied to alumina (36 g) contained in a column 300 mm long and 37 mm internal diameter. The glycosides were eluted from the column with 400 ml portions of solvent. The eluate (400 ml) thus collected was evaporated under reduced pressure and the residue weighed. The composition of the eluting solvent and the weight of the residue are shown in Table 1.

Afroside.—Fractions 16–23 (total 308 mg) from the alumina column were chromatographed on formamide-impregnated paper and showed the presence of two constituents in each of the fractions. By the intensity of the colour produced by the Raymond reaction it was estimated

that both constituents of the mixture were present in approximately the same proportions. An attempt to separate these two constituents by chromatography on alumina resulted in the original material being recovered and no separation was effected. Recrystallization of this material similarly produced no separation of the mixture, but it was obtained as square plates from methanol-water (296 mg). This mixture is referred to as *afroside* and has m.p. 258–262 °C, $[\alpha]_D^{21}$ +42±2° (*c*, 1·02 in pyridine) (Found: C, 64·6, 64·7, 64·9; H, 7·8, 7·9, 7·7; O, 27·2; OMe, 0·35; OAc, 1·6%. Calc. for $C_{29}H_{42}O_9$: C, 65·2; H, 7·9; O, 26·9; 1×OMe, 5·8; 1×OAc, 8·1%).

This compound gave positive Legal and Raymond reactions but negative Keller-Kiliani reaction. The test for the presence of carbohydrate was positive. Colour reaction with conc. H_2SO_4 was as follows (time in min): 0 yellow, 15 orange-yellow, 30 brown-orange, 60 red-brown, 120 red-brown. The total quantity of afroside isolated by chromatography on alumina of the mixed glycosides obtained from the five extractions of the plant material was 3·480 g.

Acetylafroside.—Afroside (98·4 mg), m.p. 258–262 °C, was dissolved in dry pyridine (2·0 ml) and diluted with acetic anhydride (2·0 ml). The solution was left standing at room temperature (18 °C) for 48 hr. The reaction mixture was then diluted to 4 ml with water and the acetylated material extracted with chloroform. From the chloroform solution, after washing and drying, 89 mg of colourless syrup was obtained on evaporation of the chloroform. Recrystallization of this material from methanol-water gave 73 mg of colourless crystals of *acetylafroside*, m.p. 196–198 °C, $[\alpha]_D^{18}$ −18·3±2° (*c*, 0·92 in MeOH) (Found: C, 62·2; H, 7·1; OAc, 18·5%. Calc. for $C_{35}H_{48}O_{13}$: C, 62·1; H, 7·1; 3×OAc, 18·6%).

This compound was shown to be homogeneous by chromatography on formamide-impregnated paper, using the solvent system (ii), and also on the reversed phase system using solvent system (i) (R_F=0·26).

Attempted Formation of an Oxime from Afroside.—Afroside (32 mg) was dissolved in ethanol (5 ml), hydroxylamine hydrochloride (54 mg), and sodium acetate ($CH_3CO_2Na.3H_2O$) (102 mg) in water (2 ml) were added. This mixture was refluxed for 2 hr. The solution was diluted with water (5 ml), the ethanol removed *in vacuo*, and extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulphate, evaporated, and the residue redissolved in methanol (1 ml) and transferred to a centrifuge tube. Five drops of water were added and the solution slowly evaporated. The supernatant liquid was removed from the crystals, which were then washed with aqueous methanol (9 : 1) and finally dried. Crystals (23 mg in yield), m.p. 225–227 °C, were obtained. Chromatography of this mixture on neutral alumina (1 g) gave crystalline material (16 mg), m.p. 231–234 °C, which was shown to consist of the same two constituents by chromatography on formamide-impregnated paper (solvent system (ii)). A nitrogen determination of this mixture showed the presence of 0·74% nitrogen.

Attempted Mannich Hydrolysis of Afroside.—Afroside (300 mg) was dissolved in 15 ml of methanol-chloroform (1 : 3) and evaporated under vacuum to dryness. Pure dry acetone (150 ml) was added and heated to boiling, then cooled rapidly to room temperature (18 °C), and conc. HCl (1·5 ml) added. The suspended afroside gradually dissolved. This solution was shaken at room temperature for 12 days.

The acetone was removed under vacuum, water (50 ml) being added gradually as the acetone was removed. Then methanol (50 ml) was added and the solution refluxed for 30 min. The methanol was removed by vacuum distillation and the aqueous solution crystallized out. The crystals were collected and washed repeatedly until the filtrate was neutral. Recrystallization from methanol-water gave colourless crystals of afroside B (247 mg), m.p. 256–264 °C, $[\alpha]_D^{17}$ −9·4±2° (*c*, 1·06 in MeOH) (Found: C, 65·3, 64·9; H, 8·0, 8·0%. Calc. for $C_{29}H_{42}O_9$: C, 65·2; H, 7·9%). Chromatography on formamide-impregnated paper showed this compound to be identical with one of the components of the original afroside mixture.

Acetylation of Afroside B.—Afroside B (125 mg) was dissolved in dry pyridine (2·5 ml) and acetic anhydride (2·5 ml) added. After standing at room temperature (20 °C) for 48 hr the solution was treated as for the acetylation of afroside, and recrystallization from methanol-water gave colourless crystals of acetylafroside B (110 mg), m.p. 194–205 °C, $[\alpha]_D^{20}$ −15·6±2°

(*c*, 0·96 in MeOH) (Found: C, 62·1; H, 7·6; OAc, 17·1%. Calc. for $C_{29}H_{46}O_{13}$: C, 62·1; H, 7·1; 3×OAc, 18·7%). A mixed m.p. determination with acetylafroside showed no depression. Also, acetylafroside and acetylafroside B could not be separated by chromatography on formamide-impregnated paper or on the reversed phase systems.

Saponification of Acetylafroside with Potassium Bicarbonate.—Acetylafroside (62 mg) was dissolved in methanol (7·5 ml) and diluted with an aqueous 5% potassium bicarbonate (1·5 ml) solution. The reaction mixture stood at room temperature for 12 days; most of the methanol was evaporated under vacuum at 30 °C, and the remaining aqueous methanolic solution extracted with chloroform-methanol (9 : 1, 4×10 ml). The chloroform solution after being washed with water and dried over anhydrous sodium sulphate was evaporated under vacuum to dryness. Colourless foam (43 mg) was obtained, which on recrystallization from methanol-water gave 34 mg of crystalline material, m.p. 252–258 °C, which was identified as afroside B by mixed m.p. (undepressed), and chromatography on formamide-impregnated paper.

Reduction of Afroside with Sodium Borohydride.—Afroside (97 mg) was dissolved in 10 ml of 75% dioxane-water. To this solution sodium borohydride (24 mg) in 2·4 ml of 75% dioxane-water was added over a period of 20 min. The solution then stood at room temperature (18–19 °C) for 4½ hr. The solution was just alkaline to phenolphthalein. Dilute H_2SO_4 was added drop by drop to the solution (H_2 being evolved) until it was just acid to Congo red paper. Then 6·5 ml of methanol-water (1 : 1) was added and the solution let stand at room temperature for 16 hr. (A slight precipitate appeared after the acidification, which redissolved on the addition of the aqueous methanol.) Evaporation of the methanol under vacuum caused the formation of a precipitate. This material was filtered off and the mother liquors extracted with chloroform-methanol (9 : 1). A total yield of 93 mg of white powder was obtained. This material was shown to be a boric acid complex.

Decomposition of the Boric Acid Complex.—The 93 mg of material obtained from the sodium borohydride reduction of afroside was dissolved in methanol (5 ml), and D-mannitol (0·5 g) was added. This solution was then diluted with 0·1N H_2SO_4 (5 ml) and refluxed for 30 min. The methanol was removed *in vacuo* and the compound crystallized from the aqueous acid solutions. A yield of 54 mg of colourless crystals of afrosidol was obtained, m.p. 230–235 °C, $[\alpha]_D^{24}$ +13·6±2° (*c*, 0·65 in $CHCl_3$) (Found: C, 65·3; H, 8·3%. Calc. for $C_{29}H_{44}O_9$: C, 64·9; H, 8·3%). The physical constants, mixed m.p., and chromatography on formamide-impregnated paper showed afrosidol to be different from afroside B and the other constituent of afroside.

Hydrolysis of Afrosidol with 5% Sulphuric Acid.—Afrosidol (172 mg) was dissolved in methanol (25 ml), and 10% H_2SO_4 (25 ml) in 75% aqueous methanol was added. The solution was refluxed for 5 hr, after which the methanol was removed by vacuum distillation and the hydrolysis product crystallized. A yield of 57 mg of colourless crystals was obtained, m.p. 205–210 °C, which gave positive Raymond, Legal, and tetranitromethane reactions. Recrystallization of *α-anhydroafrogenol* from acetone-methanol-water (2 : 4 : 1) raised the m.p. to 210–220 °C (amorphous), $[\alpha]_D^{20}$ +45·8±2° (*c*, 0·649 in EtOH) (Found: C, 66·4; H, 8·1%. Calc. for $C_{23}H_{34}O_6$: C, 67·95; H, 8·4%).

Extraction of the mother liquors of the hydrolysis reaction with chloroform-methanol (9 : 1) gave 62 mg of resin which after crystallization from acetone-methanol-water (2 : 4 : 1) had m.p. 208–216 °C (51 mg).

Treatment of α-Anhydroafrogenol with Periodic Acid.—Anhydroafrogenol (42·64 mg; 0·000105 g-mol) was dissolved in 10 ml of dioxane-water (1 : 1), and 0·01950M periodic acid (10 ml) solution in water was added. This solution was let stand at room temperature (22 °C) for 24 hr. Water (10 ml), 0·1N sodium arsenite (20 ml) solution, and 10% potassium iodide (1 ml) solution were added and the solution left to stand for 20 min. The sodium arsenite in excess was estimated by titration with 0·10N iodine solution. Titration after 24 hr: volume of 0·10N I_2 required = 16·80 ml. By calculation the quantity of periodic acid used in the reaction = 0·0000350 g-mol.

Acetylation of Anhydroafrogenol.—Anhydroafrogenol (68 mg) was dissolved in pyridine (2 ml), and acetic anhydride (2 ml) was added. This solution stood at room temperature for

3 days and after treating as for the acetylation of afroside, a yield of 81 mg of yellowish powder was obtained, which could not be crystallized.

The acetylated material (81 mg) was dissolved in 5 ml of a mixture of benzene-chloroform (9 : 1) and chromatographed on neutral alumina (1·5 g). The results are presented in Table 2.

The 64 mg of acetylated material obtained from the alumina column could not be crystallized. This material, acetyl-α-anhydroafrogenol, had m.p. 160–165 °C (Found : C, 67·0 ; H, 7·6 ; OAc, 14·4%. Calc. for $C_{27}H_{38}O_8$: C, 66·4 ; H, 7·3 ; 2×OAc, 15·4%).

Hydrogenation of Acetyl-α-anhydroafrogenol.—Platinum oxide (5 mg) in ethanol (2 ml) was hydrogenated and then acetylanhydroafrogenol (52 mg) dissolved in ethanol (2 ml) containing HCl (0·1 ml) was added. When the hydrogen uptake had ceased, the reaction-mixture was filtered, diluted to 10 ml with water, and then extracted with chloroform. The chloroform extract was washed with sodium bicarbonate and then water, and finally dried over anhydrous sodium sulphate. Evaporation of the chloroform gave a yield of approx. 40 mg of a pale yellow resin which could not be crystallized. This resin did not give a Raymond reaction, but gave a strong tetranitromethane reaction.

TABLE 2

PURIFICATION OF ACETYL-α-ANHYDROAFROGENOL ON ALUMINA

Fraction Number	Eluting Solvent (5 ml)	Weight of Residue (mg)	Identification by Paper Chromatography
1–5	C_6H_6 : $CHCl_3$ (9 : 1)	11	Acetylated product
6–10	C_6H_6 : $CHCl_3$ (4 : 1)	34	
11–15	C_6H_6 : $CHCl_3$ (2 : 3)	19	
16–19	C_6H_6 : $CHCl_3$ (1 : 4)	*c.* 3	—
19–25	$CHCl_3$	*c.* 10	Anhydroafrogenol

Platinum oxide (5 mg) in ethanol (2 ml) was hydrogenated, and then the material recovered from the previous experiment (50 mg), dissolved in ethanol (2 ml) containing HCl (0·1 ml) added. When the uptake of hydrogen had ceased, the reaction mixture was filtered and treated as in the previous experiment. Evaporation of the chloroform gave 43 mg of pale yellow resin which did not give a Raymond reaction, but reacted strongly with tetranitromethane. This material was used in the following oxidation experiment.

Oxidation of the Hydrogenation Product of Acetylanhydroafrogenol by Chromium Trioxide in Pyridine.—The hydrogenated product (43 mg) obtained from the previous experiment was dissolved in dry pyridine (2 ml) and chromium trioxide-pyridine complex (2 ml) added. (Prepared by the method of Poos *et al.* (1953) from chromium trioxide (43 mg) in dry pyridine (2 ml).) After standing at room temperature for 20 hr, the reaction mixture was poured into water (10 ml). The aqueous solution was extracted with chloroform, the chloroform phase being filtered through "Super-cel" to break the emulsion. The reddish orange chloroform extract, after washing with dilute H_2SO_4, sodium bicarbonate solution, and water, was dried over anhydrous sodium sulphate. This extract was evaporated to dryness under vacuum and redissolved in 5 ml of chloroform-benzene (1 : 1), and chromatographed on neutral alumina (1 g).

A colourless substance (approx. 5 mg) was obtained from fractions 3–6 (eluted by chloroform-benzene, 2 : 1 to 4 : 1), which gave a positive tetranitromethane reaction. However, there was insufficient for crystallization and constant determination.

Attempted Hydrolysis of Afroside with 0·05N H_2SO_4.—Afroside (105 mg) was dissolved in methanol (10 ml) and diluted to 20 ml with aqueous 0·1N H_2SO_4, and the solution refluxed for 30 min. (Shortly after the solution began to reflux the material precipitated.) Most of the methanol was removed by vacuum distillation and on crystallization 98 mg of colourless crystals

were recovered. This material was shown by paper chromatography on formamide-impregnated paper to be unchanged afroside. The same experiment was repeated using the 98 mg of recovered afroside in methanol (15 ml) to which was added 0·5N H_2SO_4 (5 ml), and refluxing for 1 hr. After treating as in the previous experiment, 93 mg of unchanged afroside was recovered.

Hydrolysis of Afroside with 5% H_2SO_4 in 75% Methanol-Water.—Afroside (103 mg) was dissolved in methanol (25 ml) and diluted with 25 ml of a solution of 10% H_2SO_4 in methanol-water (3 : 1). After refluxing this solution for 3 hr, the methanol was evaporated under reduced pressure, water (25 ml) being added gradually during the evaporation. On cooling, colourless crystals were deposited, which were collected and washed with water until the washings were neutral. The mother liquors were extracted with chloroform which, after washing, drying, and evaporating, gave another 6 mg of material.

Paper chromatography on formamide-impregnated paper showed the presence of a small amount of the starting material as well as the hydrolysis product. This mixture was dissolved in 5 ml of chloroform-benzene (1 : 1) and chromatographed on alumina (3·5 g). The hydrolysis product (56 mg) was eluted from the chromatography column (see Table 3). Recrystallization of the hydrolysis product from aqueous methanol gave colourless crystals (51 mg in yield) of *α-anhydroafrogenin*, m.p. 208–220 °C, $[\alpha]_D^{17}$ +60·8±2° (*c*, 0·855 in MeOH) (Found : C, 68·3 ; H, 8·0%. Calc. for $C_{23}H_{30}O_6$: C, 68·6 ; H, 7·5%. Calc. for $C_{23}H_{32}O_6$: C, 68·3 ; H, 8·0%).

This genin gave a positive reaction with tetranitromethane. When treated with concentrated H_2SO_4, the following colour changes were observed (time in min) : 0 yellow, 30 orange, 60 brown, 120 brown.

TABLE 3

PURIFICATION OF α-ANHYDROAFROGENIN ON ALUMINA

Fraction Number	Eluting Solvent (5 ml)	Weight of Residue (mg)	Identification by Paper Chromatography
1	$CHCl_3$: C_6H_6 (1 : 1)	—	—
2–8	$CHCl_3$: C_6H_6 (1 : 1 to 3 : 1)	56	Hydrolysis product
9–10	$CHCl_3$	Trace	—
12–16	$CHCl_3$: MeOH (9 : 1 to 1 : 1)	9	Afroside

Hydrogenation of Anhydroafrogenin.—Anhydroafrogenin (18·8 mg in 2 ml of ethanol) was hydrogenated at atmospheric pressure and room temperature (20 °C) in the presence of platinum oxide (2·2 mg) suspended in ethanol (0·5 ml). (The platinum catalyst was reduced before the introduction of the anhydroafrogenin.) The hydrogenation was continued until the uptake of hydrogen ceased. The solution was filtered and evaporated to dryness *in vacuo*. The white crystalline residue gave a positive reaction to tetranitromethane, but negative reactions to the Legal and Raymond colour tests.

The recovered material was redissolved in acetic acid (1·0 ml) containing 2 drops of conc. HCl and hydrogenated in the presence of platinum oxide (2 mg) in acetic acid (1·0 ml) until the uptake of hydrogen ceased. The solution was filtered, diluted to 5 ml with water, neutralized with sodium bicarbonate solution, and extracted with chloroform. The chloroform solution on evaporation gave a pale yellow oil which was redissolved in methanol, transferred to a centrifuge tube, and diluted with water until a precipitate began to form. The solution was evaporated slowly and then centrifuged. The supernatant liquid was removed with a capillary pipette and the residue washed three times with a mixture of water-methanol (5 : 1), and finally dried. This material gave negative results when tested with the Legal, Raymond, and tetranitromethane reactions.

Acetylation of α-Anhydroafrogenin.—α-Anhydroafrogenin (146 mg) was dissolved in dry pyridine (2 ml) and diluted with acetic anhydride (2 ml). After standing at room temperature (20 °C) for 48 hr, this solution was treated as in the previous acetylation reactions. After four recrystallizations from aqueous methanol 126 mg of colourless crystals of acetyl-α-anhydroafrogenin was obtained, m.p. 188–194 °C, $[\alpha]_D^{18}$ +2·5±2° (*c*, 0·81 in MeO H) (Found: C, 64·9; H, 7·7; OAc, 12·5%. Calc. for $C_{25}H_{34}O_8$: C, 64·9; H, 7·4; 1×OAc, 9·3%).

Treatment of Acetyl-α-anhydroafrogenin with Chromium Trioxide in Acetic Acid.—Acetyl-α-anhydroafrogenin (2·0 mg approx.) was dissolved in acetic acid (0·5 ml) (stable to chromium trioxide) and a 2% solution of chromium trioxide (0·5 ml) in acetic acid was added. After standing for 16 hr at room temperature, the reaction mixture turned green. A blank reaction carried out at the same time remained reddish brown. The reaction mixture was diluted with methanol-water (5 ml; 1 : 1). This solution stood at room temperature until the reduction of the chromium trioxide was complete. Extraction with chloroform gave approx. 5 mg of a neutral fraction, which gave a positive Raymond reaction, but which could not be crystallized. This material was shown to be different from the starting material by chromatography on formamide-impregnated paper.

Treatment of Acetyl-α-anhydroafrogenin with Chromium Trioxide-Pyridine Complex in Pyridine.—Acetyl-α-anhydroafrogenin (32 mg) was dissolved in dry pyridine (0·5 ml) and a chromium trioxide-pyridine complex (0·5 ml approx.) was added. (This complex was prepared by the method of Poos *et al.* (1953). The chromium trioxide-pyridine complex contained 64 mg of chromium trioxide dissolved in 1 ml of dry pyridine.) The reaction mixture was thoroughly mixed and allowed to stand at room temperature overnight, before being treated in the manner described in the oxidation of acetyl-α-anhydroafrogenol.

Evaporation of the chloroform extract gave approx. 3 mg of a yellow oil which could not be separated from a sample of the starting material when chromatographed on formamide-impregnated paper.

IV. Acknowledgments

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V. References

Bally, P. R. O., Mohr, K., and Reichstein, T. (1951).—*Helv. Chim. Acta* **34**: 1740.
Brockman, H., and Schodder, H. (1941).—*Chem. Ber.* **74**: 73.
Cardwell, H. M. E., and Smith, S. (1954).—*J. Chem. Soc.* **1954**: 2012.
Casteel, H. W., and Wender, S. H. (1953).—*Analyt. Chem.* **25**: 508.
Cole, A. R. H. (1952).—*J. Chem. Soc.* **1952**: 4969.
Cole, A. R. H., Jones, R. N., and Dobriner, K. (1952).—*J. Amer. Chem. Soc.* **74**: 5571.
Fürst, A., Kuhn, H. H., Scotoni, R. Jr., and Günthard, Hs. H. (1952).—*Helv. Chim. Acta* **35**: 951.
Heftmann, E., and Levant, A. J. (1952).—*J. Biol. Chem.* **194**: 703.
Hunger, A., and Reichstein, T. (1952*a*).—*Helv. Chim. Acta* **35**: 429.
Hunger, A., and Reichstein, T. (1952*b*).—*Helv. Chim. Acta* **35**: 1073.
Hurst, E. (1942).—"The Poison Plants of N.S.W." p. 326. (Snelling Printing Press: Sydney.)
Jensen, K. B. (1953).—*Acta Pharm. Tox. Kbh.* **9**: 99.

JONES, R. N., and HERLING, F. (1954).—*J. Org. Chem.* **19**: 1252.
JONES, R. N., HUMPHRIES, P., HERLING, F., and DOBRINER, K. (1951).—*J. Amer. Chem. Soc.* **73**: 3215.
KATZ, A. (1953).—*Helv. Chim. Acta* **36**: 1344.
KELLER, M., and REICHSTEIN, T. (1949).—*Helv. Chim. Acta* **32**: 1607.
KLYNE, W. (1954).—"Progress in Stereochemistry." Vol. 1. p. 167. (Butterworth: London.)
MANNICH, C., and SIEWERT, G. (1942).—*Chem. Ber.* **75**: 737.
MEREDITH, P., and SAMMONS, H. G. (1952).—*Analyst* **77**: 416.
POOS, G. I., ARTH, G. E., BEYLER, R. E., and SARETT, L. H. (1953).—*J. Amer. Chem. Soc.* **75**: 422.
RAYMOND, W. D. (1938).—*Analyst* **63**: 478.
RAYMOND, W. D. (1939).—*Analyst* **64**: 113.
SCHINDLER, O., and REICHSTEIN, T. (1951).—*Helv. Chim. Acta* **34**: 108.
SCHINDLER, O., and REICHSTEIN, T. (1954).—*Helv. Chim. Acta* **37**: 1163.
SILBERMAN, H., and THORP, R. H. (1953).—*J. Pharm. Pharmacol.* **4**: 438.
SVENSEN, A. B., and JENSEN, K. B. (1950).—*Pharm. Acta Helv.* **25**: 241.
THORP, R. H., and WATSON, T. R. (1953).—*Aust. J. Exp. Biol. Med. Sci.* **31**: 529.
TSCHESCHE, R., GRIMMER, G., and SEEHOFER, F. (1953).—*Chem. Ber.* **86**: 1239.
WATSON, T. R., and WRIGHT, S. E. (1954).—*Chem. & Ind.* **1954**: 1178.
WATT, J. M., and BREYER-BRANDWIJK, M. G. (1932).—"The Medicinal and Poisonous Plants of Southern Africa." p. 149. (Livingstone: Edinburgh.)
WEBB, L. J. (1948).—Guide to the medicinal and poisonous plants of Queensland. C.S.I.R.O. Aust. Bull. No. 232.
WINTERSTEINER, O., and MOORE, M. (1943).—*J. Amer. Chem. Soc.* **65**: 1513.
ZAFFARONI, A., BURTON, R. B., and KEUTMANN, E. H. (1949).—*J. Biol. Chem.* **177**: 109.
ZAFFARONI, A., BURTON, R. B., and KEUTMANN, E. H. (1950).—*Science Lond.* **111**: 6.
ZAFFARONI, A., BURTON, R. B., and KEUTMANN, E. H. (1951).—*J. Biol. Chem.* **188**: 763.

THE ALKALOIDS OF *ECHIUM PLANTAGINEUM* L.

I. ECHIUMINE AND ECHIMIDINE

By C. C. J. CULVENOR*

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Summary

Two new alkaloids, echiumine, $C_{20}H_{31}O_6N$, and echimidine, $C_{20}H_{31}O_7N$, have been isolated from *Echium plantagineum* L. Both have a retronecine nucleus esterified on the 7-hydroxyl group with angelic acid. The main esterifying acid of echiumine is trachelanthic acid and of echimidine is 2-methyl-2,3,4-trihydroxypentane-3-carboxylic acid, identical with the esterifying acid of heliosupine. The structure of heliosupine, a diastereoisomer of echimidine, has been confirmed.

I. INTRODUCTION

Echium plantagineum L. is an escaped garden plant which has spread over large areas of southern Australia. As its common names, Paterson's Curse and Salvation Jane, indicate, it is a weed in good pastures while on poor country it is considered as a reserve fodder for stock. *E. plantagineum* has occasionally been suspected of being poisonous to stock but a feeding experiment in N.S.W. gave negative results (Hurst 1942) and the plant is generally regarded as harmless to animals. Recently, however, it has been found to cause fatalities among sheep at Albury, N.S.W. (Bull *et al.* 1956), and pathological examination of affected animals revealed liver damage of the type produced by *Heliotropium europaeum* L. (Bull 1955). Like *Heliotropium*, *Echium* belongs to the family Boraginaceae, and consequently the presence of pyrrolizidine alkaloids was strongly indicated. Traces of non-crystalline alkaloid have previously been found in *E. vulgare* L. (Manske 1936).

Preliminary assays showed that the total alkaloid content of *E. plantagineum* varies widely, being 0·07 per cent. for an Adelaide sample and 0·50 and 0·17 per cent. for Albury samples taken in different years. Paper chromatography of the total crude base, about 80 per cent. of which occurs as *N*-oxide, showed spots of R_F 0·67, 0·58, 0·46, and 0·31. Assay of the reduced base from the second Albury sample by the partition column procedure of Culvenor, Drummond, and Price (1954) gave the following results: R_F 0·67, 0·021 per cent.; R_F 0·58, 0·053 per cent.; R_F 0·46, 0·048 per cent.; R_F 0·41, 0·014 per cent.; and R_F 0·31, 0·012 per cent. In all samples of *E. plantagineum* studied so far, the base R_F 0·58 has been the one present in greatest quantity. In the first Albury sample, the base R_F 0·46 did not appear at all. The present paper reports the isolation and structural determination of the bases R_F 0·67 and 0·58, which have been named echiumine and echimidine respectively.

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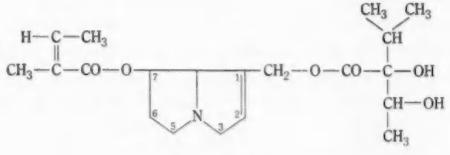
A convenient method for the isolation of echiumine was found in a modified countercurrent distribution of the total base between chloroform and dilute hydrochloric acid, which also removed most of the dark coloured impurities which accompany the crude base. This dark colour reforms when base in a gummy condition is stored for a period of weeks without rigid exclusion of light and air. Partition chromatography on buffered glass powder provides a fairly good separation of the constituents on a small-scale assay column but larger columns were not so successful (presumably because of poorer packing or heavier loading). When the base of R_F 0·46 is absent as in the first Albury sample, echimidine is easily separated from the bases of lower R_F by extraction from aqueous sodium carbonate with carbon tetrachloride, the remaining bases being extracted only with chloroform. In the presence of the base R_F 0·46, this separation procedure was of little value. The best method for purification of echiumine and echimidine from the base R_F 0·46 was by chromatography on silica gel. From silica gel moistened with water, only echiumine is readily eluted by chloroform. From silica gel moistened with concentrated aqueous ammonium sulphate, echimidine is eluted rapidly by chloroform and the bases of lower R_F only very slowly.

Echiumine, $C_{20}H_{31}O_6N$, was obtained in crystalline form, m.p. 99–100 °C, $[\alpha]_D^{18}$ +14·4° (ethanol), and formed a crystalline picrate, m.p. 131–132 °C. Hydrolysis of echiumine gave rise to angelic acid, trachelanthic acid, and retronecine while hydrogenolysis with a platinum catalyst produced trachelanthic acid and a base, $C_{13}H_{23}O_2N$, identical with the 7-(2′-methylbutyryl)-retronecanol obtained by hydrogenolysis of echimidine (see below). Echiumine therefore has structure I, being retronecine esterified on the 7-hydroxyl group with angelic acid and on the 1-hydroxymethyl group with trachelanthic acid.

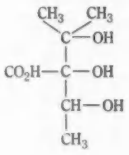
Echimidine was obtained only as a clear glass but gave with some difficulty a crystalline picrate, $C_{20}H_{31}O_7N.C_6H_3O_7N_3$, and a crystalline *N*-oxide, $C_{20}H_{31}O_8N$. Base recovered from the pure picrate had $[\alpha]_D^{18}$ +13·4° (ethanol). The empirical formula, $C_{20}H_{31}O_7N$, indicated for echimidine was confirmed by the degradation results. From the alkaline hydrolysis of echimidine, acetone, angelic acid, and retronecine were isolated. On hydrogenation with a platinum catalyst, 3 moles of hydrogen were consumed; the basic product was an oil, $C_{13}H_{23}O_2N$, which gave retronecanol on hydrolysis and in view of the isolation of angelic acid from echimidine, must be 7-(2′-methylbutyryl)retronecanol. The esterifying acid liberated by hydrogenolysis could not be extracted from aqueous solution with chloroform but was isolated by evaporating the acidified reaction mixture to dryness and extracting the residue with hot chloroform. The acid so obtained was a non-crystallizable glass, R_F 0·50 in butanol-acetic acid, forming a crystalline brucine salt, $C_7H_{14}O_5.C_{23}H_{26}O_4N_2$. The formula $C_7H_{14}O_5$ for this acid is in agreement with the formula $C_{20}H_{31}O_7N$ for echimidine. On periodate oxidation, this acid consumed 2·0 moles of periodate giving acetone, acetaldehyde, and oxalic acid as products.

It follows from these results that the second esterifying acid of echimidine is 2-methyl-2,3,4-trihydroxypentane-3-carboxylic acid (II). An acid of this structure was first reported by Menshikov and Petrova (1952) as the esterifying

acid of macrotomine, an alkaloid of the boraginaceous plant, *Macrotomia echioides* Boiss. This acid, named macrotomic acid, decomposed during hydrolysis of the alkaloid and since it was esterified with the saturated compound, trachelanthamidine, it could not be obtained from macrotomine by hydrogenolysis. The empirical formulae of the alkaloid and its hydrolysis base showed that the acid must be $C_7H_{14}O_5$, and when the alkaloid yielded acetone, acetaldehyde, and oxalic acid on periodate oxidation, structure III was assigned to macrotomine. Macrotomic acid has also been reported as an esterifying acid of the



(I)



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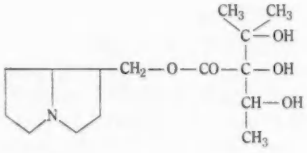

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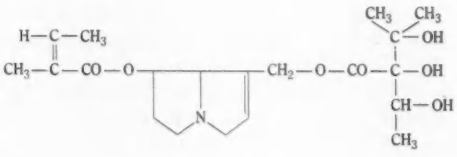

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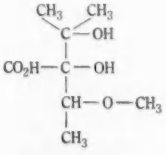

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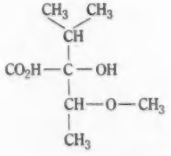

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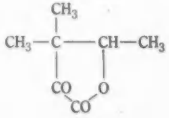

(VII)

base, heliosupine, from *Heliotropium supinum* L. (Denisova, Menshikov, and Utkin 1953). Although this alkaloid is susceptible to hydrogenolysis, the acid was again not isolated and structure IV was assigned to heliosupine on the grounds of hydrolysis to angelic acid and heliotridine, and periodate oxidation to acetone, acetaldehyde, and oxalic acid. The Russian authors considered the esterifying acid of heliosupine to be identical with macrotomic acid but since the possibility of diastereoisomerism was not excluded, this conclusion is not justified.

It is clear, however, that heliosupine is a diastereoisomer of echimidine which is also represented by structure IV. Because of the possible identity of the acids in these two alkaloids, heliosupine has been isolated from *H. supinum*,

growing in Victoria, and re-examined. Our sample of heliosupine has the same properties as the base described by Denisova, Menshikov, and Utkin (1953), and likewise could not be obtained crystalline. It occurs in the Victorian plant together with supinine and two other alkaloids and its isolation from this source will be described in a later paper. On hydrolysis it yielded angelic acid, heliotridine, and acetone, while on hydrogenolysis it gave 7-(2′-methylbutyroxy)-heliotridane and a glassy acid which was proved by optical rotation and mixed melting point of the brucine salts to be identical with the acid from echimidine. Echimidine therefore differs from heliosupine only in the configuration of the C-atom in position 7 of the pyrrolizidine ring.

Since it was first isolated from echimidine and its possible identity with macrotomic acid cannot at present be decided, the trihydroxy acid here described is provisionally named echimidinic acid. An attempt has been made to relate it stereochemically to lasiocarpic acid (V) which differs structurally in that the secondary hydroxyl is methylated. Unfortunately, although heliotric acid (VI) can be demethylated in good yield with 48 per cent. hydrobromic acid, the same process causes decomposition of lasiocarpic acid to steam volatile and tarry materials only. The reaction of lasiocarpic acid with concentrated hydrochloric acid, which gives similar results but without tar formation, has been investigated in some detail and will be considered in a subsequent paper. It may be stated here that the main identified products are acetaldehyde, dimethylpyruvic acid, and ββ-dimethyl-α-keto-γ-valerolactone (VII). Even in the early stages no trace of echimidinic acid could be detected in the reaction mixture, and this acid was in fact found to be more readily decomposed by concentrated hydrochloric acid than is lasiocarpic acid. The products isolated were again acetaldehyde and dimethylpyruvic acid, the decomposition obviously being similar to that of lasiocarpic acid. This provides confirmation of the structure of echimidinic acid but not evidence of stereochemical similarity of the two acids.

II. Experimental

Analyses were carried out in the C.S.I.R.O. Microanalytical Laboratory. Melting points are corrected. Unless otherwise stated, the solvent used for paper chromatography was the upper phase resulting from shaking butanol with an equal volume of acetic acid (5%).

(*a*) *Assays.*—The methods used for total alkaloid and detailed assays were those previously described (Culvenor, Drummond, and Price 1954; Culvenor and Smith 1955).

(*b*) *Extraction of Crude Alkaloid.*—The dried, milled plant was extracted with methanol either in a Soxhlet apparatus or by percolation with cold solvent. The two methods were found to give the same yield of alkaloid. Methanol was removed from the extract under reduced pressure and the residue was extracted with dilute sulphuric acid. The aqueous acid solution was reduced by stirring with zinc dust and additional 70% sulphuric acid to make the solution 1N with respect to acid. The crude alkaloid was obtained by making alkaline to phenolphthalein with ammonia, and extracting with chloroform. In working up the first Albury sample, from which the base of R_F 0·46 was absent, a worthwhile separation was achieved at this stage by preliminary extraction of the basified solution with carbon tetrachloride. This solvent removed echiumine and echimidine only, and subsequent chloroform extraction gave the base of R_F 0·38. In later samples, which contained the base of R_F 0·46, no separation was achieved in this way.

(*c*) *Isolation of Echiumine by Distribution between Chloroform and Dilute Hydrochloric Acid.*—In a normal countercurrent distribution of the crude base between chloroform (mobile phase)

and 0·25N HCl (stationary phase), echiumine moved with the chloroform more rapidly than did the other bases but too slowly for the procedure to be a convenient method of isolation. When total crude base was dissolved in chloroform and extracted with successive inferior amounts of N/8 HCl echiumine was concentrated in the later fractions. The two effects were combined to give an effective separation procedure as in the following example. Crude base (7·1 g) was distributed in separatory funnels between N/10 HCl (50 ml) and chloroform (100 ml) with addition of sodium carbonate (0·025 g; approx. equivalent to 5 ml of N/10 acid) to No. 1 separatory funnel after each separation of phases. In this way, excess base was maintained in the first 3 funnels, the object being to displace all echiumine beyond No. 3 funnel. Eleven funnels were used and base finally recovered from each with chloroform after addition of excess sodium carbonate. The products are shown in Table 1.

Retreating the main concentrates gave fractions corresponding to 5 and 6 in Table 1 which crystallized. These fractions were extracted several times with hot light petroleum (b.p. 60–70 °C) and the extracts concentrated and seeded. The resulting crystals were recrystallized from a small volume of acetone to give essentially pure echiumine.

TABLE 1

MODIFIED DISTRIBUTION OF CRUDE BASE BETWEEN CHLOROFORM AND DILUTE HYDROCHLORIC ACID

Fraction	Weight (g)	R_F Values
1	0·11	0·55, 0·42, 0·33 (weak)
2	2·14	0·58 (strong), 0·42, 0·32 (weak)
3	2·05	0·67 (weak), 0·58, 0·42 (weak)
4	1·67	0·67 (strong), 0·55, 0·41 (very weak)
5	0·65	0·67
6	0·21	0·67
7	0·22	0·73, 0·63 (strong)
8	0·03	0·67
9	0·02	—
10	0·15	—
11	0·27	—

(*d*) *Echiumine* forms long needles from light petroleum containing 15% acetone, m.p. 99–100 °C, $[\alpha]_D^{18}$ +14·4° (*c*, 2·02 in ethanol) (Found: C, 63·5; H, 8·5; N, 3·9; O, 25·5%; equiv. wt., 385. Calc. for $C_{20}H_{31}O_6N$: C, 63·0; H, 8·2; N, 3·7; O, 25·2%; equiv. wt., 381). *Echiumine picrate*, prepared by mixing the base with a small excess of picric acid in ethanol, removing the solvent, and crystallizing from 50% aqueous ethanol, formed yellow prisms, m.p. 131–132 °C (Found: C, 51·3; H, 5·6; N, 8·9; O, 34·1%. Calc. for $C_{26}H_{34}O_{13}N_4$: C, 51·2; H, 5·6; N, 9·2; O, 34·1%).

(*e*) *Chromatography on Silica Gel and the Isolation of Echimidine.*—Two types of column packing were used. The first was silica gel moistened with water, and from this column only echiumine was readily eluted by chloroform. The remaining bases came through in a single peak with chloroform-10% methanol. This served to purify echiumine and to provide mixed base free from echiumine. The isolation of echimidine was effected with a column of silica gel moistened with concentrated aqueous ammonium sulphate. Silica gel (24 g) was mixed in a mortar with aqueous ammonium sulphate (16 ml of solution made up from 20 g sulphate in 25 ml water) and packed into a 40 × 1·2 cm column. The alkaloid (mixed bases not containing echiumine; equivalent to 24·8 ml of 0·01N acid) was applied in chloroform solution and elution continued with this solvent. Fractions of 5 ml were collected and titrated with 0·01N *p*-toluenesulphonic acid. Titres and R_F values of products are shown in Table 2. Most of the echimidine

(R_F 0·59) was obtained in a pure state, but the base of R_F 0·47 was contaminated with residual amounts of echimidine brought out by chloroform–2% ethanol. In subsequent runs with larger columns, much larger amounts of mixed bases were applied than would ordinarily be used with this type of column since only the first eluted component was required. Thus with an 80 × 2·4 cm column packed with silica gel (160 g) and aqueous ammonium sulphate (65 ml; the maximum amount which leaves the silica gel free-flowing and permits packing under gravity), 10·6 g base was applied and 4·3 g echimidine obtained pure. Application of 20·5 g mixed base to a similar column gave 8·2 g of pure echimidine.

(*f*) *Purification of Echimidine and Echimidine Picrate.*—The purest fractions of echimidine could not be induced to crystallize even after keeping for 6 months in a refrigerator. The picrate was obtained crystalline with difficulty but once seeds were available essentially pure echimidine was readily converted into crystalline picrate. From hot water, the *picrate* separated as yellow needles, m.p. 142–143 °C (Found: C, 50·1; H, 5·6; N, 8·7; O, 35·9%. Calc. for $C_{26}H_{34}O_{14}N_4$: C, 49·8; H, 5·5; N, 8·9; O, 35·8%). On recovery from this picrate, echimidine formed a clear gum, $[\alpha]_D^{18}$ +13·4° (*c*, 1·64 in ethanol).

TABLE 2

ELUTION OF ALKALOID FROM SILICA GEL-AMMONIUM SULPHATE

Fraction	Eluting Solvent	Titre (ml)	R_F Values
3–14	$CHCl_3$	0·83	—
15–24	,,	2·74	0·57
25–30	,,	2·74	0·59
31–42	,,	3·18	0·59
43–56	,,	2·87	0·59
57–64	$CHCl_3$–2% EtOH	4·26	0·59 (strong) 0·47 (weak)
65–91	,,	5·94	0·60, 0·48
92–114	,,	2·42	0·60, 0·48

The echimidine should be essentially pure before conversion into picrate or the picrate m.p. will be low and difficult to raise by recrystallization. The m.p. of one sample could not be improved beyond 132–133 °C; the analytical data for this sample were still reasonably good (Found: C, 50·1; H, 5·4; N, 8·5%), and base recovered from it agreed in specific rotation (+14·2°) with that from picrate of highest melting point.

(*g*) *Echimidine N-Oxide.*—The base (2·3 g) was dissolved in a little ethanol and added to aqueous hydrogen peroxide (100 vol.; 15 ml). After 24 hr, excess peroxide was destroyed with manganese dioxide, the ethanol removed under reduced pressure, and the aqueous solution washed with chloroform and then evaporated to dryness under reduced pressure. Rubbing the product with acetone gave a grey powder (1·01 g), m.p. 159–161 °C. Recrystallization from acetone-methanol gave the *N*-oxide as prisms, m.p. 165 °C (decomp.) (Found: C, 58·5; H, 7·8; N, 3·4%. Calc. for $C_{29}H_{31}O_8N$: C, 58·0; H, 7·6; N, 3·4%). On keeping in a stoppered tube for 3–4 weeks, the *N*-oxide decomposed into a black tar.

(*h*) *Hydrolysis of Echiumine.*—Echiumine (0·5 g) was refluxed for 2 hr in 2N NaOH (10 ml) and ethanol (5 ml) with a slow stream of hydrogen bubbling through the solution and then being passed through a solution of 2,4-dinitrophenylhydrazine sulphate. Only traces of a gummy deposit formed in the latter solution. Removal of ethanol from the reaction mixture, acidification of the residue, and extraction with chloroform gave a partly crystalline oil (0·21 g), R_F 0·76 in butanol-acetic acid, R_F 0·29, 0·32 in butanol-ammonia. In these solvents, trachelanthic acid has R_F 0·76 and 0·33 respectively; angelic acid has R_F 0·29 in the latter but is too volatile to

form a spot in the former solvent. Extraction of the mixed acids with light petroleum gave angelic acid, which formed long needles from water, m.p. 42–43 °C, mixed m.p. 43–44 °C. The petrol-insoluble acid was sublimed *in vacuo* and crystallized from light petroleum to give trachelanthic acid as small rosettes, m.p. and mixed m.p. 91–92 °C.

The basic product was isolated by concentrating the aqueous solution to 5–10 ml, adding solid sodium hydroxide (5 g), and extracting many times with chloroform. Recrystallization of the product (0·15 g) from acetone gave retronecine as colourless prisms, m.p. 116–117 °C, mixed m.p. 117–119 °C (Found : C, 61·9 ; H, 8·5 ; N, 9·3%. Calc. for $C_8H_{13}O_2N$: C, 62·0 ; H, 8·4 ; N, 9·0%).

(*i*) *Hydrogenolysis of Echiumine.*—When shaken with hydrogen and reduced platinum oxide catalyst in dilute hydrochloric acid, echiumine (0·5 g) absorbed 94 ml (3·0 moles) hydrogen. After filtering, the solution was made alkaline, and extracted with chloroform to give an oil (0·35 g), which on distillation from a bulb tube at 0·4 mm, came over mainly at bath temp. 70 °C. This main fraction R_F 0·64 was redistilled and then had $[\alpha]_D^{28}$ —124° (*c*, 1·18 in ethanol) (Found : C, 68·5 ; H, 10·3 ; N, 6·2%. Calc. for $C_{13}H_{23}O_2N$: C, 69·3 ; H, 10·3 ; N, 6·2%). The low C content is possibly due to presence of a small amount of impurity since distillation on this scale does not permit effective fractionation. From ethanol, the base formed a picrate, m.p. 205–206 °C, undepressed on admixture with the picrate of the corresponding base from echimidine (see below).

The residual aqueous solution was acidified to Congo red and extracted in a separatory funnel with chloroform to give an oil (0·04 g), R_F 0·76, which did not crystallize. Continuous chloroform extraction of the solution gave a gum (0·16 g) which crystallized completely. Recrystallization from benzene-light petroleum gave trachelanthic acid as rosettes, m.p. and mixed m.p. 93–94 °C, $[\alpha]_D^{20}$ +3·0±1·0° (*c*, 1·00 in ethanol) (Found : C, 52·0 ; H, 8·6 ; O, 38·9%. Calc. for $C_7H_{14}O_4$: C, 51·8 ; H, 8·7 ; O, 39·5%). Trachelanthic acid from supinine had m.p. 94–95 °C, $[\alpha]_D^{18}$ +2·3° (*c*, 1·7 in ethanol) (Culvenor 1954).

(*j*) *Hydrolysis of Echimidine.*—Echimidine (1·63 g) was boiled for 2 hr in 2N sodium hydroxide (20 ml) under partial reflux, a small amount of aqueous distillate being allowed to pass over into alcoholic 2,4-dinitrophenylhydrazine. A precipitate which formed in the latter solution was identified as acetone 2,4-dinitrophenylhydrazone, m.p. and mixed m.p. 123–125 °C. The reaction solution was acidified to Congo red, filtered, and extracted three times with benzene and then four times with chloroform. The benzene extracts gave an oil (0·34 g) which crystallized readily and separated from water in long needles, m.p. 43–45 °C, unchanged on admixture with angelic acid (Found : C, 60·0 ; H, 8·2%. Calc. for $C_5H_8O_2$: C, 60·0 ; H, 8·0%). The chloroform extracts produced only a few mg of gum, R_F 0·55 in butanol-acetic acid.

The residual aqueous solution was concentrated *in vacuo* to approx. 10 ml, sodium hydroxide (6 g) added, and the solution extracted with chloroform. The product (0·56 g) crystallized from acetone as prisms, m.p. 117–119 °C, $[\alpha]_D^{17}$ +50·7° (*c*, 1·62 in ethanol) (Found : C, 62·1 ; H, 8·3 ; N, 8·9%. Calc. for $C_8H_{13}O_2N$: C, 61·9 ; H, 8·4 ; N, 9·0%). A mixture with retronecine (lit. m.p. 121–122 °C, $[\alpha]_D$ +50·2° in ethanol) melted at 117–118 °C, a mixture with heliotridine below 85 °C.

(*k*) *Hydrogenolysis of Echimidine.*—When shaken with hydrogen and platinum oxide catalyst in dilute hydrochloric acid, echimidine (2·0 g) absorbed 3·0 moles hydrogen. The resulting dark red solution was made alkaline to phenolphthalein and extracted with chloroform to give a basic oil (1·51 g), the main constituent of which had R_F 0·68, and lesser constituents 0·53, 0·47, and 0·39. When distilled from a bulb tube at 0·5 mm, the greater part of the oil came over at bath temp. 75 °C. This fraction, 7-(2′-methylbutyryl)retronecanol, after redistillation, had $[\alpha]_D^{20}$ —129° (*c*, 1·60 in ethanol), R_F 0·64 (Found : C, 69·9 ; H, 10·4 ; N, 6·5%. Calc. for $C_{13}H_{23}O_2N$: C, 69·3 ; H, 10·3 ; N, 6·2%). It formed a picrate, crystallizing from ethanol in yellow needles, m.p. 204–206 °C (Found : C, 50·5 ; H, 5·6 ; N, 12·5%. Calc. for $C_{19}H_{26}O_9N_4$: C, 50·2 ; H, 5·8 ; N, 12·3%). When the base was refluxed with aqueous ethanolic sodium hydroxide for 1·5 hr, the ethanol removed, and the solution extracted with chloroform, a clear

gum was obtained, which crystallized spontaneously. On distillation from a bulb tube at 0·5 mm and bath temp. 90–120 °C, it condensed as prisms, m.p. 93–94 °C. A mixture with authentic retronecanol had m.p. 93–94 °C. For determination of the optical rotation the total distillate, which contained some impurities in the early and late portions, was used; this had $[\alpha]_D^{20}$ —108° (*c*, 1·755 in ethanol). Retronecanol is quoted as having m.p. 95–96 °C, $[\alpha]_D^{28}$ —91° (ethanol).

The residual aqueous solution from the hydrogenation yielded no product when made acid to Congo red and extracted with chloroform. The main esterifying acid was isolated only by evaporating the acidified aqueous solution to dryness at room temp. in an air stream, finally in a desiccator, and extracting the residue several times with hot chloroform. The acid was a colourless glass (0·73 g) R_F 0·55, which although soluble in hot benzene precipitated from this solvent on cooling as a gum or gel. It gave a crystalline *brucine salt*, forming prisms from ethanol, m.p. 209–210 °C (Found: C, 63·1; H, 7·3; N, 4·7; O, 25·0%. Calc. for $C_{39}H_{46}O_9N_2$: C, 62·9; H, 7·0; N, 4·9; O, 25·2%). When recovered from this salt by dissolving in water, making the solution alkaline, removing brucine with chloroform, acidifying with hydrochloric acid, evaporating to dryness and extracting with chloroform, *echimidinic acid* (*2-methyl-2,3,4-trihydroxypentane-3-carboxylic acid*) was a colourless glass, $[\alpha]_D^{20}$ +16·4° (*c*, 1·55 in ethanol).

(*l*) *Periodate Oxidation of Echimidinic Acid.*—Echimidinic acid (10 ml of aqueous solution containing 0·00785 g/ml, standardized against N/100 sodium hydroxide), and periodic acid (10 ml of aqueous solution containing 0·0403 g/ml) were mixed and kept at room temp. After 18 hr and also after 23 hr, 2·0 molar proportions of periodic acid had been consumed. Reaction products were identified as follows: (i) *Acetaldehyde:* Nitrogen was bubbled slowly through part of the reaction solution (5 ml) and then through an ethanolic solution of dimedone containing a drop of piperidine. Addition of water to the latter solution gave a precipitate which crystallized from aqueous methanol in prisms, m.p. 142–143 °C, undepressed on admixture with authentic acetaldehyde dimedone compound. (ii) *Acetone:* The reaction solution (5 ml) was made alkaline with barium hydroxide and treated with ammoniacal silver nitrate to destroy acetaldehyde. When precipitation of silver was complete, the mixture was filtered and the filtrate distilled. The first few ml of distillate gave a 2,4-dinitrophenylhydrazone which after recrystallization from ethanol had m.p. 123–124 °C, mixed m.p. 124–125 °C with authentic acetone dinitrophenylhydrazone. (iii) *Oxalic acid:* The remaining reaction solution was extracted thoroughly with ether. Evaporation of the extracts gave a white solid with the solubilities of oxalic acid. Sublimation at 80 mm pressure and bath temp. 100–150 °C gave a sublimate, m.p. 116–120 °C. Authentic oxalic acid hydrate similarly treated gave a sublimate, m.p. 102–103 °C. Apparently, the oxalic acid from echimidinic acid had lost part of its water of crystallization. The amount available was too small to attempt recrystallization or complete dehydration but the reaction product behaved in exactly the same way as authentic oxalic acid in the colour reaction described by Martin (1955) as being specific for oxalic acid.

(*m*) *Heliosupine.*—The isolation of heliosupine from Victorian *Heliotropium supinum* will be described in a later paper. The alkaloid was obtained as a clear gum and purified through the picrate. Our picrate had the characteristic property reported by Denisova, Menshikov, and Utkin (1953) of crystallizing as a hydrate, m.p. 102–106 °C, and being converted into a gum on drying *in vacuo* at 60–80 °C. Base recovered from this picrate had $[\alpha]_D^{20}$ —4·3° (*c*, 5·10 in ethanol). Heliosupine was stated by Denisova, Menshikov, and Utkin to be optically inactive.

(*n*) *Hydrogenolysis of Heliosupine and Isolation of Echimidinic Acid.*—When heliosupine (0·55 g) was shaken in dilute hydrochloric acid with hydrogen and platinum oxide catalyst, 3·1 moles of hydrogen was absorbed. The products were isolated as in the reduction of echimidine above. The resulting base (0·32 g) was a mixture of four constituents, of R_F 0·69, 0·58, 0·52, 0·23, with the first predominating. This main component distilled from a bulb tube at 0·2 mm and bath temp. 65 °C. On redistillation it had $[\alpha]_D^{17}$ +0·9±1° (*c*, 1·10 in ethanol) (Found: C, 70·3; H, 10·5; N, 6·3%. Calc. for $C_{12}H_{23}O_3N$: C, 69·3; H, 10·3; N, 6·2%). The picrate crystallized from ethanol in needles, m.p. 157–159 °C, and did not depress the m.p. of the picrate of 7-(2′-methylbutyroxy)heliotridane derived from lasiocarpine.

The acid was a colourless glass (0·22 g), R_F 0·54, but its brucine salt crystallized from ethanol in prisms, m.p. 209–211 °C, undepressed on admixture with the brucine salt of echimidinic acid (Found: C, 63·1; H, 7·1; N, 4·9%. Calc. for $C_{36}H_{40}O_9N_2$: C, 62·9; H, 7·0; N, 4·9%). The pure acid recovered from this brucine salt had $[\alpha]_D^{20}$ +17·5° (*c*, 1·12 in ethanol).

III. Acknowledgments

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IV. References

Bull, L. B. (1955).—*Aust. Vet. J.* **31**: 33.

Bull, L. B., Dick, A. T., Keast, J. C., and Edgar, G. (1956).—*Aust. J. Agric. Res.* **7**: 281.

Culvenor, C. C. J. (1954).—*Aust. J. Chem.* **7**: 287.

Culvenor, C. C. J., Drummond, L. J., and Price, J. R. (1954).—*Aust. J. Chem.* **7**: 277.

Culvenor, C. C. J., and Smith, L. W. (1955).—*Aust. J. Chem.* **8**: 556.

Denisova, S. I., Menshikov, G. P., and Utkin, L.M. (1953).—*C.R. Acad. Sci. U.R.S.S.* **93**: 59.

Hurst, Evelyn (1942).—"The Poison Plants of New South Wales." (Snelling Printing Press: Sydney.)

Manske, R. H. F. (1936).—*Canad. J. Res.* **14**: 6.

Martin, S. M. (1955).—*Chem. & Ind.* **1955**: 427.

Menshikov, G. P., and Petrova, M. F. (1952).—*J. Gen. Chem. Moscow* **22**: 1457.

THE ALKALOIDS OF *SENECIO JACOBAEA* L.

III. THE STRUCTURE OF JACONECIC ACID

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Summary

Oxidation of jaconecic acid by nitric acid yields four acid products, which have been separated by a combination of partition chromatography on silica gel, and paper chromatography. Two of these fragments have been identified as αβ-dimethylmalic acid and oxalic acid, whilst the other two are dibasic acids having the formulae $C_9H_{14}O_6$ and $C_9H_{12}O_7$. These findings support the structure previously assigned to jaconecic acid.

I. Introduction

In Parts I (Bradbury 1954) and II (Bradbury and Willis 1956) of this series, jaconecic acid as found in the alkaloid jacobine was provisionally assigned the structure I, which incorporates several features not commonly found in the C_{10} "necic" acids (Adams 1953; Warren 1955). These are (i) the presence of a substituted glutaric, rather than an adpic acid structure, (ii) a glycidic acid grouping, and (iii) a less common mode of linking of two isoprene units (Schinz and Bourquin 1942).

To obtain further evidence on the validity of the proposed structure, a study was made of the oxidation of jaconecic acid with nitric acid. When jaconecic acid was heated at 100 °C with dilute nitric acid, and the excess mineral acid and water removed under vacuum, a thick viscous oily residue remained. Paper chromatography, using butanol saturated with 5 per cent. acetic in water, and spraying the dried paper with bromocresol green revealed the presence of four acids. These were cleanly separated on a column of silica gel with 0·5N sulphuric acid as the stationary phase and chloroform and mixtures of chloroform and acetone for the moving phase. By titration of aliquots of each fraction and application to paper similar fractions could be combined. In this way three solid acids and one liquid acid were obtained chromatographically pure (see Section III).

The compound with the lowest R_F value which gave a streak rather than a discrete spot on paper was identified as oxalic acid.

The acid with R_F 0·59 solidified on standing and when recrystallized had m.p. 100 °C, was optically active ($[\alpha]_D$ −3·6°), and analysed for the formula $C_6H_{10}O_5$. It formed a monoacetate, was dibasic, and contained two carbon-methyl groups. A possible structure is that of αβ-dimethylmalic acid and one racemate (m.p. 151 °C) of αβ-dimethylmalic acid was synthesized by a modi-

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fication of the procedure of Michael and Tissot (1891, 1892) and Anschutz (1928). The R_F values of both samples were identical, and the brucine salts showed no depression in melting point when admixed, but the *p*-phenylphenacyl and *p*-bromophenacyl derivatives showed depression. An attempt to resolve the brucine salt of the racemate by crystallization from water to constant rotation, and recovery of the acid proved unsuccessful. However, verification that the compound from the oxidation of jaconecic acid was indeed αβ-dimethylmalic acid was obtained by its dehydration to dimethylmaleic anhydride which was identical with a specimen similarly prepared from the synthetic racemate.

The compound of R_F 0·35 had $[\alpha]_D$ +20·3° but could not be obtained crystalline. Its dimethyl ester could however be purified by distillation, b.p. 130 °C/1 mm, $[\alpha]_D$ +25·6°, and its analysis agreed with the formula $C_{11}H_{18}O_6$. The infra-red spectrum showed a weak band at 1647 cm^{-1} probably due to a double bond, and carbonyl absorption at 1749 and 1801 cm^{-1}. The presence of a hydroxyl group was indicated by a band at 3442 cm^{-1} and a shoulder at 3634 cm^{-1}. The presence of a ketone group was indicated by weak absorption in the ultraviolet ($\varepsilon_{max.}$ 501 at 252 mμ; Morton and Rosney 1926) and another weak band ($\varepsilon_{max.}$ 547 at 222 mμ) was present. The presence of a carbonyl group was conclusively established by the preparation of a 2,4-dinitrophenylhydrazone of the free acid.

When the dimethyl ester was hydrolysed with excess alkali the recovered acidic material showed three spots on paper. The main one corresponded in R_F value with the starting material, and another with that of αβ-dimethylmalic acid. The third spot which was very faint had a higher R_F value than αβ-dimethylmalic acid.

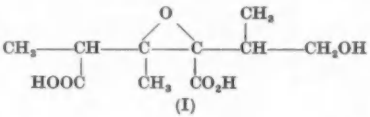
The fourth compound of R_F 0·48, m.p. 181 °C, $[\alpha]_D$ +18·2° was a dibasic acid analysing according to the formula $C_9H_{12}O_7$. It formed a nicely crystalline di-*p*-bromophenacyl derivative, m.p. 191 °C, which showed carbonyl absorption in the infra-red band at 1702, 1747, 1770, and 1801 cm^{-1}, but no hydroxyl absorption.

The preparation of αβ-dimethylmalic acid by the procedure of Michael and Tissot (loc. cit.) which involves the gradual addition of hydrochloric acid to a mixture of an ethereal solution of ethyl methylacetoacetate and solid potassium cyanide, and allowing the mixture to stand for a week, was found to be unreliable in the hands of Auwers and von Campenhausen (1896). These authors recommended a second treatment with potassium cyanide and hydrochloric acid. In the present work more satisfactory results were achieved by reacting ethyl methylacetoacetate with liquid hydrogen cyanide, in the presence of catalytic amounts of potassium cyanide. However, even by this procedure the yield of crystalline αβ-dimethylmalic acid obtained on hydrolysis of the crude cyanohydrin was rather low. This might have been due to the reversibility of the cyanohydrin addition reaction, and to the fact that two racemates of αβ-dimethylmalic acid could have been formed. However, an examination of the mother liquors from the crystallization of the αβ-dimethylmalic acid by chromatography on silica gel failed to reveal the presence of a second racemate. Instead, traces of methylacetoacetic acid cyanohydrin, m.p. 102 °C, and trimethylmalic acid,

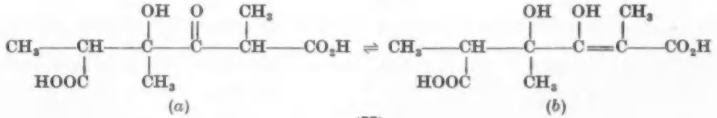
m.p. 161 °C, together with a further amount of the same racemate of αβ-dimethylmalic acid were obtained. The cyanohydrin appears to be new, but the trimethylmalic acid which has previously been prepared by Auwers and von Campenhausen (loc. cit.) must arise from ethyl dimethylacetoacetate present as an impurity in the ethyl methylacetoacetate.

II. Discussion

The identification of αβ-dimethylmalic acid (III) as one of the products of oxidation of jaconecic acid has satisfactorily accounted for 6 out of the 10 carbon atoms. The formation of two other acids $C_9H_{14}O_6$ and $C_9H_{12}O_7$ from jaconecic acid by the loss of one carbon atom, can only be accounted for by decarboxylation, since a terminal methylene group, the only other feasible alternative, has previously been shown to be absent. Decarboxylation has been shown to occur merely by heating jaconecic acid, and it is apparent that this occurs with nitric acid also at the carbon atom containing an α-oxygen substituent (Bradbury and Willis loc. cit.). In the compound $C_9H_{14}O_6$ the formation of a keto-group which was not originally present in jaconecic acid, supports this contention. The possibility that this keto-group can enolize is shown by the absorption at 222 mμ in the ultraviolet absorption spectrum, and by the weak absorption in the infra-red in the double bond region. This band in the ultraviolet also indicates that the double bond is conjugated with a carboxyl group, which of necessity must be that formed from the oxidation of the primary hydroxyl group. The dimethyl ester shows hydroxyl absorption at 3492 cm^{-1}. Both the acids $C_9H_{14}O_6$ and $C_9H_{12}O_7$ are dibasic and, therefore, a carboxyl group has been formed from a primary hydroxyl group originally present in jaconecic acid.

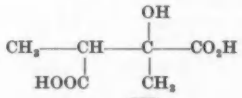


(I)



(a) (b)

(II)



(III)

In proposing a structure for the dibasic acid $C_9H_{14}O_6$ it is accepted as implicit that αβ-dimethylmalic acid can be derived from it as indicated by paper chromatographic analysis of the products of alkaline hydrolysis of the ester. It is most probable that the carboxyl group in αβ-dimethylmalic acid with the α-hydroxyl substituent is derived from the keto-group in the dibasic

acid $C_9H_{14}O_6$, and that this carbon atom held the carboxyl group (capable of decarboxylation) in jaconecic acid. The three carbon atoms to be added to αβ-dimethylmalic acid to form the C_9 acid must include a carboxyl group originally derived from a primary hydroxyl group in jaconecic acid, and this can only lead to the formulae II (*a*) and (*b*) for the dibasic acid $C_9H_{14}O_6$.

III. Experimental

All melting points are corrected. Microanalyses were carried out in the C.S.I.R.O. Microanalytical Laboratory.

Oxidation of Jaconecic Acid with Nitric Acid.—Jaconecic acid (16·8 g), concentrated nitric acid (40 ml), and water (40 ml) were warmed on a boiling-water bath for a few minutes until a vigorous reaction set in, and then the flask was let stand in a beaker of cold water until the reaction had subsided. The excess nitric acid and water were removed under vacuum on the water-bath, and the residue was allowed to stand in a vacuum desiccator over solid sodium hydroxide for 2 weeks until a constant weight of 14·6 g was obtained. This was chromatographed on silica gel (1 kg) moistened with 0·5N sulphuric acid (100 ml), in a column 5×90 cm, using chloroform and mixtures of chloroform and acetone for elution. Fractions of 50 ml were collected, and 1 ml aliquots from each were evaporated to dryness and titrated with 0·01N sodium hydroxide to the phenolphthalein end-point. Those fractions having a significant titre were tested by paper chromatography for homogeneity using the ascending technique. Butanol saturated with 5% acetic acid in water was used as solvent and the papers were run for 16 hr, dried, and sprayed with saturated aqueous bromocresol green which gave yellow spots on a blue background. The results are presented in Table 1.

Table 1

Chromatography of the Oxidation Products of Jaconecic Acid on Silica Gel

Fraction No.	R_F Value	Combined Wt. of Fractions (g)	Remarks
1–83	—	1·76	Oily
84–97	0·48	1·38	Solid
98–101	0·35, 0·48	0·62	Solid
102–127	0·35	4·05	Oily
128–137	0·59	2·52	Solid
138–147	0·59, 0·20	1·63	Solid

Acid $C_9H_{12}O_7$.—Fractions 84–97 were combined and evaporated to dryness to give a residue (1·38 g) which solidified on standing in a vacuum desiccator over solid sodium hydroxide. When recrystallized from ethyl acetate-light petroleum prisms, m.p. 181 °C (softening at a lower temperature), formed, $[\alpha]_D^{27}$ +18·2° (*c*, 2·306 in water) (Found: C, 46·7; H, 5·2; CH_3C, 20·0%; equiv. (by titration), 119. Calc. for $C_9H_{12}O_7$: C, 46·5; H, 5·2; $3\times CH_3C$, 19·3%; equiv., 116).

Di-p-bromophenacyl Derivative.—The acid (0·61 g) above was neutralized with 0·2N sodium hydroxide and the solution evaporated nearly to dryness. *p*-Bromophenacyl bromide (1·32 g) was added and ethanol to effect solution at the boiling point. It was refluxed for 3 hr, when a sparingly soluble crystalline derivative separated. When recrystallized from acetone it formed colourless needles, m.p. 191 °C (Found: C, 48·2; H, 3·5; Br, 25·4%; mol. wt. (Rast), 557. Calc. for $C_{25}H_{22}O_9Br_2$: C, 47·9; H, 3·5; Br, 25·5%; mol. wt., 626).

Acid $C_6H_{14}O_6$.—Fractions 102–127 were combined and evaporated to dryness to give a residue (4·05 g) which after standing for some weeks in a desiccator over solid sodium hydroxide did not solidify, and attempts to crystallize it from various solvents failed. It had $[\alpha]_D^{26}$ +20·3° (*c*, 2·168 in water).

Dimethyl Ester of Acid $C_9H_{14}O_6$.—A part of fraction 102–127 (3·5 g) was treated with diazomethane in ether from nitrosomethylurea (10·3 g), after standing for 64 hr, the ether and excess diazomethane were removed. The residue was distilled and the middle fraction, b.p. 129–132 °C/1 mm, $[\alpha]_D^{24}$ +25·6° (*c*, 1·564 in ethanol), n_D^{22} 1·4547, D_{22}^{22} 1·185 (Found: C, 53·1; H, 7·1; OCH_3, 24·2; CH_3C, 12·3%. Calc. for $C_{11}H_{18}O_6$: C, 53·6; H, 7·4; OCH_3, 25·2; $2\times CH_3C$, 12·2%). Light absorption: $\varepsilon_{max.}$ 547 at 2220 Å; 501 at 2520 Å. The infra-red spectrum showed carbonyl absorption at 1749 and 1801 cm^{-1}, hydroxyl absorption at 3442 and 3634 cm^{-1}, and a weak band at 1647 cm^{-1}.

The dimethyl ester (0·29 g), sodium hydroxide (0·2 g), water (2 ml), and ethanol (2 ml) were heated on a boiling-water bath for 1 hr. After standing a further 16 hr at room temperature the acidified solution was extracted with ether. The residue left on removal of the ether gave three spots when tested by paper chromatography. The main spot represented unchanged starting material, another had the same R_F value as pure optically active αβ-dimethylmalic acid, and a very faint spot occurred at a higher R_F value.

2,4-Dinitrophenylhydrazone.—The dimethyl ester above (0·13 g), 2,4-dinitrophenylhydrazine (0·10 g), 0·1N hydrochloric acid (4·7 ml), and ethanol to effect solution, were refluxed for 2 hr and then evaporated to dryness. A reddish oil remained which did not crystallize. 2N hydrochloric acid (20 ml) was then added and the solution kept at 100 °C for 2 hr. On standing, a yellow solid separated which when recrystallized from benzene had m.p. 148–149 °C (Found: C, 45·1; H, 4·8; N, 13·6%. Calc. for $C_{15}H_{18}O_9N_4$: C, 45·2; H, 4·6; N, 14·1%).

αβ-Dimethylmalic Acid.—Fractions 128–137 when evaporated gave a residue (2·52 g) which solidified on keeping over solid sodium hydroxide. When recrystallized from ethyl acetate-light petroleum colourless prisms, m.p. 100 °C, $[\alpha]_D^{24}$ −3·6° (*c*, 2·206 in water) were obtained (Found: C, 44·6; H, 6·5; CH_3C, 17·4%; equiv. (by titration), 82. Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·2; $2\times CH_3C$, 18·5%; equiv., 81).

Brucine Salt.—The above acid (0·11 g) in water (5 ml) was added to anhydrous brucine (0·46 g), the solution concentrated to about 1 ml, filtered hot, and allowed to crystallize. Colourless needles were obtained which when recrystallized again from water had m.p. 201 °C, $[\alpha]_D^{26}$ −17·1° (*c*, 1·75 in water) (Found: C, 62·8; H, 6·8; N, 5·8%. Calc. for $C_{52}H_{62}O_{12}N_4.2H_2O$: C, 63·3; H, 6·7; N, 5·7%).

Acetyl Derivative.—The acid (0·35 g) was refluxed with acetyl chloride (5 ml) for 2 hr, and the excess acetyl chloride removed. The residue yielded colourless prisms, m.p. 155 °C, from acetone-light petroleum on long standing (Found: C, 47·6; H, 5·8; CH_3CO, 22·1%; equiv. (by titration), 103. Calc. for $C_8H_{12}O_6$: C, 47·1; H, 5·9; CH_3CO, 21·1%; equiv., 102).

Di-p-phenylphenacyl Derivative.—The acid (0·12 g) was neutralized with 0·1N sodium hydroxide and *p*-phenylphenacyl bromide (0·4 g) added with ethanol to effect solution. The solution was refluxed for 2 hr, concentrated, and the solid derivative recrystallized from ethanol yielding colourless granules, m.p. 188 °C (Found: C, 74·9; H, 5·3; O, 20·2%; mol. wt. (Rast), 533. Calc. for $C_{34}H_{30}O_7$: C, 74·2; H, 5·5; O, 20·3%; mol. wt., 551).

Di-p-bromophenacyl Derivative.—This was prepared in a similar manner, and gave colourless needles, m.p. 151–152 °C, from ethanol (Found: C, 48·0; H, 3·8; Br, 27·5%. Calc. for $C_{32}H_{26}O_7Br_2$: C, 47·5; H, 3·6; Br, 28·7%).

Dimethylmaleic Anhydride.—The acid (1·96 g) was heated to 250 °C when water began to distil. When this had ceased the residue was distilled under reduced pressure, b.p. 153–154 °C/130 mm. The distillate solidified and when sublimed at 100 °C/0·5 mm gave colourless prisms, m.p. 94 °C (Found: C, 57·0; H, 5·0%. Calc. for $C_6H_6O_3$: C, 57·1; H, 4·8%). Anschutz (loc. cit.) reports m.p. 95–96 °C for this compound.

Oxalic Acid.—Fractions 138–147 on evaporation gave a solid (1·63 g) which when recrystallized from water gave colourless prisms, m.p. 100–102 °C, undepressed on admixture with hydrated oxalic acid. When thoroughly dry it had m.p. 191 °C (Found: equiv. (by titration), 47. Calc. for $C_2H_2O_4$: equiv., 45·02).

dl-αβ-*Dimethylmalic Acid.*—Ethyl methylacetoacetate (40·4 g) cooled to 0 °C was added to liquid hydrogen cyanide (32 g) cooled to 0 °C, followed by a few crystals of solid potassium cyanide. After standing at room temperature for 64 hr, the brown coloured mixture was treated with conc. hydrochloric acid (50 ml) with cooling. After a few minutes a vigorous reaction set in, and the excess hydrogen cyanide was evolved. On cooling, the solid ammonium chloride which separated was filtered, and the filtrate was heated with a further quantity of conc. hydrochloric acid (20 ml) for 2 hr. Water was added to the cooled solution until the ammonium chloride just dissolved and the mixture was continuously extracted with ether for 5 hr. A yellow oil (40·2 g) remained after removal of the ether which from ethyl acetate-light petroleum yielded *dl*-αβ-dimethylmalic acid (10 g). A portion recrystallized several times from ethyl acetate-light petroleum gave colourless prisms, m.p. 150–151 °C (Found: C, 44·7; H, 6·2; CH_3C, 17·0%; equiv. (by titration), 81. Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·2; $2\times CH_3C$, 18·5%; equiv., 81).

Brucine Salt.—*dl*-αβ-Dimethylmalic acid (1·62 g), anhydrous brucine (7·89 g), and water (30 ml) were heated on a water-bath until solution was effected. On cooling, colourless needles of the brucine salt were obtained. Two more recrystallizations from water gave a product with constant rotation $[\alpha]_D^{26}$ —21·1° (*c*, 2·562 in water), m.p. 203–204 °C, undepressed on admixture with the specimen obtained from the oxidation of jaconecic acid (Found: C, 61·1; H, 6·3; N, 5·8; O, 26·7%. Calc. for $C_{52}H_{62}O_{13}N_4.4H_2O$: C, 61·0; H, 6·9; N, 5·5; O, 26·6%). After drying at 100 °C/0·1 mm over phosphorus pentoxide for 6 hr (Found: C, 63·7; H, 6·9; N, 5·9%. Calc. for $C_{52}H_{62}O_{13}N_4.2H_2O$: C, 63·3; H, 6·7; N, 5·7%). The brucine salt (3·4 g) was dissolved in (1 : 1) conc. hydrochloric acid, water (30 ml), and continuously extracted with ether for 5 hr. Removal of the ether gave the acid (0·54 g) $[\alpha]_D \pm 0$ (*c*, 2·15 in water).

Cinchonidine Salt.—*dl*-αβ-Dimethylmalic acid (1·41 g) and cinchonidine (2·56 g) were dissolved in ethanol (20 ml), an equal amount of water added, the ethanol distilled off, and the solution allowed to crystallize. Recrystallization of the product from water gave colourless needles, m.p. 213 °C (decomp.), $[\alpha]_D^{22}$ —96·3° (*c*, 1·09 in 50% ethanol) (Found: C, 69·1; H, 7·5; N, 7·1%. Calc. for $C_{44}H_{54}O_7N_4.H_2O$: C, 68·7; H, 7·3; N, 7·3%).

Di-p-*phenylphenacyl Derivative.*—Prepared in the usual way it gave colourless crystals (from ethanol), m.p. 185 °C, depressed on admixture with the same derivative from the oxidation of jaconecic acid (Found: C, 74·0; H, 5·3%; mol. wt. (Rast), 531. Calc. for $C_{34}H_{30}O_7$: C, 74·2; H, 5·5%; mol. wt., 551).

Di-p-*bromophenacyl Derivative.*—Prepared as above and recrystallized from ethanol it gave colourless needles, m.p. 147 °C, depressed on admixture with the same derivative from the natural product (Found: C, 48·2; H, 3·3; Br, 28·1%. Calc. for $C_{22}H_{20}O_7Br_2$: C, 47·5; H, 3·6; Br, 28·7%).

Dimethylmaleic Anhydride.—This was prepared as described above, and was obtained as colourless prisms, m.p. 94 °C, undepressed on admixture with the specimen obtained from the natural product.

Chromatography of the Residue from the Crystallization of dl-αβ-*Dimethylmalic Acid.*—Portion (11·4 g) of this residue was chromatographed on silica gel using the same quantities and procedure as before. The results are presented in Table 2.

Methylacetoacetic Acid Cyanhydrin.—Fractions 112–117 when combined and evaporated to dryness gave a solid (0·53 g) which on recrystallization from ethyl acetate-light petroleum gave colourless prisms, m.p. 102 °C (Found: C, 50·4; H, 6·2; N, 9·8; CH_3C, 19·1%. Calc. for $C_6H_9O_3N$: C, 50·3; H, 6·3; N, 9·8; $2\times CH_3C$, 21·1%).

Trimethylmalic Acid.—Fractions 119–127 when combined and evaporated to dryness gave a solid (0·60 g) which when recrystallized from ethyl acetate-light petroleum gave colourless prisms, m.p. 161 °C (Found: C, 47·9; H, 7·0; CH_3C, 15·2%; equiv. (by titration), 87. Calc.

for $C_7H_{12}O_5$: C, 47·7; H, 6·9; 2×CH_3C, 17·1%; equiv., 88). Auwers and von Campenhausen (loc. cit.) report m.p. 160 °C for this compound.

Fractions 134–151 when evaporated to dryness gave an oil (6·26 g) which when recrystallized from ethyl acetate-light petroleum gave a further quantity of *dl*-αβ-dimethylmalic acid.

TABLE 2

CHROMATOGRAPHY OF αβ-DIMETHYLMALIC ACID RESIDUES ON SILICA GEL

Fraction No.	R_F Value	Weight of Fraction (g)	Remarks
1–111	—	1·28	Oily, discarded
112–117	—	0·53	M.p. 100–102 °C
119–127	0·69	0·60	M.p. 161 °C
134–151	0·60	6·26	αβ-Dimethylmalic acid

Decarboxylation of Jaconecic Acid.—Jaconecic acid (1·27 g) was heated at 230–250 °C in a stream of dry carbon dioxide-free air, and the gases passed through an absorption train. The "Carbosorb" tube showed an increase in weight of 0·27 g (theory for 1 mol CO_2: 0·24 g). A sample of the residue when tested by paper chromatography showed one strong spot at R_F 0·89, but no spot for jaconecic acid. When chromatographed on silica gel no solid compound could be isolated.

IV. Acknowledgments

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V. References

Adams, R. (1953).—*Angew. Chem.* **65**: 433.
Anschutz, R. (1928).—*Liebigs Ann.* **461**: 172.
Auwers, K., and von Campenhausen, G. F. (1896).—*Ber. dtsch. chem. Ges.* **29**: 1544.
Bradbury, R. B. (1954).—*Chem. Ind. Lond.* **1954**: 1022.
Bradbury, R. B., and Willis, J. B. (1956).—*Aust. J. Chem.* **9**: 258.
Michael, A., and Tissot, G. (1891).—*Ber. dtsch. chem. Ges.* **24**: 2544.
Michael, A., and Tissot, G. (1892).—*J. prakt. Chem.* [2] **46**: 298.
Morton, R. A., and Rosney, W. C. V. (1926).—*J. Chem. Soc.* **1926**: 706.
Schinz, H., and Bourquin, J. P. (1942).—*Helv. Chim. Acta* **25**: 1599.
Warren, F. L. (1955).—*Fortschr. Chem. org. Naturst.* **12**: 198.

SYNTHESIS OF LONG-CHAIN HYDROXY KETONES FROM HYDROXY ACIDS

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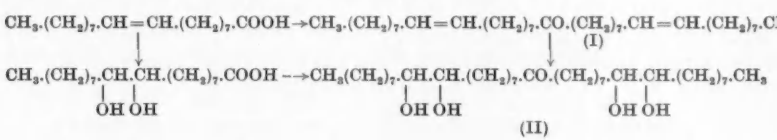
Summary

The acyl chlorides derived from *threo-* and *erythro*-dihydroxystearic acid and -dihydroxybehenic acid, in which the alcohol groups are unreactive, are converted into ketene dimers by reaction with triethylamine and thence to long-chain tetrahydroxy ketones. The ketones are apparently homogeneous but yet presumably consist of mixtures of stereoisomers.

Certain other hydroxy acids in which the hydroxyl groups are more reactive may also be used to prepare ketones if they are first acetylated.

I. INTRODUCTION

The ketones prepared from oleic, elaidic, and other unsaturated long-chain acids react with performic acid to give tetrahydroxy derivatives (Hatt and Lamberton 1955). This is a stereo-specific reaction which takes place by *trans* addition to a double bond. Oleone (I), for example, yields *threo*-9,10-*threo*-26,27-tetrahydroxypentatriacontan-18-one (II) and elaidone the corresponding *erythro-erythro*-form having a much higher melting point.



These tetrahydroxy ketones may be conveniently prepared by an alternative route. The hydroxyl groups in the *threo-* and *erythro*-forms of 9,10-dihydroxystearic (III) are unreactive and it is unnecessary to protect them in preparing the acyl chlorides, which are then converted into the *threo-* and *erythro*-forms of II by the method of Sauer (1951). An advantage of this procedure is that it avoids lengthy purification of the starting material; an ordinary commercial grade of oleic acid may be used to prepare *threo*-9,10-dihydroxystearic acid of a high degree of purity and this in turn gives a ketone requiring little purification. Similarly, a crude sample of elaidic acid is sufficient for the preparation of *erythro*-9,10-dihydroxystearic acid, which is sparingly soluble in organic solvents and readily purified by recrystallization. On the other hand, for the preparation of oleone it is necessary to start with pure oleic acid.

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The *threo*- and *erythro*-13,14-dihydroxybehenic acids, prepared from erucic and brassidic acid respectively, likewise yield the *threo-threo*- and *erythro-erythro*-forms of 9,10,34,35-tetrahydroxytritetracontan-22-one. The tetrahydroxy ketones are readily characterized by the formation of oximes and by oxidation to keto dibasic acids. Periodic acid oxidation of II gives nonanal and a crystalline aldehyde, presumably 9-ketoheptadecanedial, which on further oxidation is converted into 9-ketoheptadecanedioic acid.

Bounds, Linstead, and Weedon (1953) have shown that *threo*- and *erythro*-9,10-dihydroxystearic acids undergo the Kolbe reaction, giving the *threo-threo*- and *erythro-erythro*-forms of tetratriacontan-9,10,25,26-tetrol. The same tetrols were also prepared by hydroxylation of *cis-cis*- and *trans-trans*-9,25-tetratriacontadiene, obtained by anodic synthesis from oleic and elaidic acids respectively. These authors point out that by either of the two synthetic routes the same *threo-threo*- (or, with appropriate changes in starting materials, *erythro-erythro*-) product is obtained. But the *threo-threo*- and the *erythro-erythro*-tetrols are each a mixture of diastereoisomers, containing a (±)-compound formed by symmetrical coupling of enanthiomorphs, and a *meso*-compound formed by crossed coupling.

This also applies to the two forms of the tetrahydroxy ketone (II) prepared from *threo*- and *erythro*-9,10-dihydroxystearic acid and they presumably also consist of a (±)- and a *meso*-compound. The apparently identical products obtained by the action of performic acid on oleone and elaidone are also equimolar mixtures of (±)- and a *meso*-form resulting from *trans* addition to the double bonds.

The tetrahydroxy ketones are readily reduced by the Wolff-Kishner method to tetrols differing from those obtained by Bounds, Linstead, and Weedon (1953) from anodic coupling in that they have an odd-numbered carbon chain and an odd number of methylene groups separating the two α-glycol groups.

To prepare ketones by Sauer's (1951) method from a long-chain acid containing an isolated secondary hydroxyl group, or in which one of the hydroxyl groups of the 1,2-glycol is primary, protection of the hydroxyl groups is needed. The acetates derived from 12-hydroxystearic acid and 10,11-dihydroxyhendecanoic acid may be used to prepare 7,29-dihydroxypentatriacontan-18-one and 1,2,20,21-tetrahydroxyheneicosan-11-one respectively. The latter substance is also obtained by hydroxylation of 1,20-heneicosadien-11-one with performic acid.

II. Experimental

Microanalyses were made by the C.S.I.R.O. Microanalytical Laboratory. Melting points are all corrected.

(*a*) *Materials Used.*—(i) *threo*-9,10-Dihydroxystearic acid was prepared from commercia oleic acid and performic acid (Swern *et al.* 1945). It melted at 93–94 °C after several recrystallizations from ethyl acetate.

(ii) *erythro*-9,10-Dihydroxystearic acid was most conveniently prepared as follows: Commercial oleic acid was elaidinized, using sodium nitrite-nitric acid, and the crude elaidic acid was crystallized several times from ethanol. It was then free from *cis*-acid but contained some

TABLE 1

ANALYSES AND MELTING POINTS OF TETRAHYDROXY KETONES, THEIR OXIMES AND TETROLS

Ketone	Melting Point (°C)	Melting Point of Oxime (°C)	Analysis of Oxime (%)
(i) *threo*-9,10-*threo*-26,27-Tetrahydroxypentatriacontan-18-one ..	117–118	96–98	Found : N, 2·2 Calc. for $C_{35}H_{71}O_5N$: N, 2·4
(ii) *erythro*-9,10-*erythro*-26,27-Tetrahydroxypentatriacontan-18-one	167	133–134	Found : C, 71·9 ; H, 12·4 ; N, 2·8 Calc. for $C_{35}H_{71}O_5N$: C, 71·7 ; H, 12·2 ; N, 2·4
(iii) *threo*-9,10-*threo*-34,35-Tetrahydroxytritetracontan-22-one ..	119–120	99–100	Found : C, 74·2 ; H, 12·6 ; N, 2·4 Calc. for $C_{43}H_{87}O_5N$: C, 74·0 ; H, 12·6 ; N, 2·0
(iv) *erythro*-9,10-*erythro*-34,35-Tetrahydroxytritetracontan-22-one	163–164	131–132	Found : C, 74·3 ; H, 12·7 ; N, 2·0 Calc. for $C_{43}H_{87}O_5N$: C, 74·0 ; H, 12·6 ; N, 2·0
(v) 1,2,20,21-Tetrahydroxyheneicosan-11-one	123–124	83	Found : C, 64·5 ; H, 11·0 ; N, 3·8 Calc. for $C_{21}H_{43}O_5N$: C, 64·7 ; H, 11·1 ; N, 3·6
(vi) *7,29-Dihydroxypentatriacontan-18-one**	106–108	87–88	Found : C, 76·5 ; H, 12·9 ; N, 2·6 Calc. for $C_{35}H_{71}O_3N$: C, 76·0 ; H, 12·8 ; N, 2·5
Tetrol			**Analysis of Tetrol (%)**
(i) Pentatriacontan-*threo*-9,10-*threo*-26,27-tetrol	114–115		Found : C, 75·8 ; H, 13·1 Calc. for $C_{35}H_{72}O_4$: C, 75·5 ; H, 13·0
(ii) *Pentatriacontan*-erythro-*9,10*-erythro-*26,27-tetrol*	164–165		Found : C, 75·5 ; H, 12·9 Calc. for $C_{35}H_{72}O_4$: C, 75·5 ; H, 13·0
(iii) *Tritetracontan*-threo-*9,10*-threo-*34,35-tetrol*	116–117		Found : C, 77·0 ; H, 13·1 Calc. for $C_{43}H_{88}O_4$: C, 77·2 ; H, 13·3
(iv) *Tritetracontan*-erythro-*9,10*-erythro-*34,35-tetrol*	162–163		Found : C, 77·4 ; H, 13·0 Calc. for $C_{43}H_{88}O_4$: C, 77·2 ; H, 13·3
(v) *Heneicosa-1,2,20,21-tetrol*	146–147		Found : C, 70·2 ; H, 12·3 Calc. for $C_{21}H_{44}O_4$: C, 70·0 ; H, 12·3
(vi) *Pentatriacontan-7,29-diol*	105–106		Found : C, 80·3 ; H, 13·9 Calc. for $C_{35}H_{72}O_2$: C, 80·1 ; H, 13·8

* Found : C, 77·9 ; H, 12·9%. Calc. for $C_{35}H_{70}O_3$: C, 78·1 ; H, 13·1% ; $[\alpha]_D = 0°$ (chloroform).

saturated acids. The crude elaidic acid was then hydroxylated as in (i) and the product after several recrystallizations from ethyl acetate melted at 129–130 °C.

(iii) *threo*-13,14-Dihydroxybehenic acid was prepared from pure erucic acid as in (i), m.p. 99–100 °C.

(iv) *erythro*-13,14-Dihydroxybehenic acid was prepared from pure brassidic acid as in (i), m.p. 129–130 °C.

(v) 10,11-Dihydroxyhendecanoic acid was prepared from freshly distilled undecylenic acid by hydroxylation with performic acid. It melted at 84–85 °C, after recrystallization from aqueous-ethanol (Swern *et al.* 1945).

(vi) 12-Hydroxystearic acid was prepared from hydrogenated castor oil acids. The crude acid was converted into its methyl ester, repeatedly crystallized from light petroleum and the pure acid recovered by saponification, m.p. 78–79 °C.

(*b*) *Preparation of the Ketones.*—(i) The ketones from Section II (*a*) (i)-(iv) were prepared by the same method ; the following example is typical : To a suspension of *threo*-9,10-dihydroxystearic acid (15·8 g) in dry benzene, a slight excess of thionyl chloride or oxalyl chloride was added and the mixture refluxed for 1 hr. The benzene and unreacted thionyl chloride or oxalyl chloride were then distilled and dry ether (70 ml) added to dissolve the residue. The subsequent procedure was the same as described already (Hatt and Lamberton 1955). During the decomposition of the ketene dimer with aqueous ethanolic alkali the crude ketone separated in a crystalline condition and after heating (4 hr) the mixture was diluted with water and the crystals were filtered from the hot solution to avoid the separation of soaps. After washing with hot water, the ketone was dried and recrystallized from dioxan. *threo*-9,10-*threo*-26,27-Tetrahydroxypentatriacontan-18-one melted at 118–119 °C and the m.p. was not depressed by admixture with the product obtained by treating oleone with performic acid. Yield, 8·1 g (Found : C, 73·8 ; H, 12·5%. Calc. for $C_{35}H_{70}O_5$: C, 73·6 ; H, 12·4%).

The m.p.'s of the tetrahydroxy ketones prepared from the *trans-trans*-dienones were noticeably higher than those previously reported (see Table 1). It was suspected that the differences in m.p.'s were due to incomplete hydroxylation of the dienones by the method of the previous paper and when, following the method of Bounds, Linstead, and Weedon (loc. cit.), the hydroxylation of the dienones was repeated in a mixture of formic acid, chloroform, and acetone, which gives a homogenous solution, tetrahydroxy ketones of these higher m.p.'s were obtained.

(ii) 10,11-Dihydroxyhendecanoic acid and 12-hydroxystearic acid were both acetylated before preparing the ketones.

10,11-Dihydroxyhendecanoic acid was heated for 5 hr in acetic anhydride-pyridine mixture. After shaking with water to decompose unchanged acetic anhydride, the oily product was extracted with light petroleum and distilled under reduced pressure (b.p. 164–165 °C/1·5 mm). The acetylated acid was obtained as a colourless oil, which was converted into the acyl chloride by heating with oxalyl chloride in dry benzene. The ketonization was carried out as in the previous example. When the ketene dimer was heated with ethanolic alkali, the acetyl groups were also removed, and the crystalline tetrahydroxy ketone separated out when the mixture was diluted with water. The product was filtered off and recrystallized from methanol. 1,2,20,21-Tetrahydroxyheneicosan-11-one melted at 123–124 °C. Yield, 60% of theoretical, based on the weight of acetylated acid (Found : C, 67·5 ; H, 11·3%. Calc. for $C_{21}H_{42}O_5$: C, 67·3 ; H, 11·3%).

The same ketone was also prepared by the action of performic acid on 1,20-heneicosadien-11-one, prepared by ketonization of undecylenic acid (Hatt and Lamberton 1955). M.p. and mixed m.p. with a specimen of the material prepared above 123–124 °C.

(*c*) *Oxidation.*—As reported in the earlier paper, *threo*-9,10-*threo*-26,27-tetrahydroxypentatriacontan-18-one was oxidized by potassium periodate in dilute sulphuric acid. This oxidation was repeated and it was found that after the nonanal was removed by steam distillation, the non-volatile material crystallized on cooling. It was dried and on recrystallization from light petroleum gave colourless prisms, m.p. 61–62 °C (Found : C, 72·2 ; H, 10·7%. Calc. for $C_{17}H_{30}O_3$: C, 72·3 ; H, 10·6%).

This substance gave strong aldehyde tests and was very readily oxidized by potassium permanganate in acetone to 9-ketoheptadecanedioic acid, m.p. and mixed m.p. with an authentic specimen, 112–113 °C (Found : C, 65·1 ; H, 9·7%. Calc. for $C_{17}H_{30}O_5$: C, 65·0 ; H, 9·6%).

(*d*) *Oximes.*—The tetrahydroxy ketones have so small a solubility in ethanol that this solvent is unsuited for oximation. The reaction proceeds readily in a mixture of equal volumes of ethanol and pyridine. The following preparation typifies the method used. *threo*-9,10-*threo*-26,27-Tetrahydroxypentatriacontan-18-one (0·5 g) and hydroxylamine hydrochloride were refluxed in this solvent (10 ml) for 1½ hr. Dilution with water gave the crystalline oxime. It crystallized from methanol in colourless prisms, m.p. 96–98 °C. The properties of the oximes so prepared are listed in Table 1.

(*e*) *Tetrols.*—Wolff-Kishner reduction of the ketones was carried out with anhydrous hydrazine in diethylene glycol. To complete the reaction, heating was continued for 20 hr. In each instance the mixture was diluted with water and the crystalline product filtered and recrystallized from ethanol or dioxan. Pentatriacontan-*threo*-9,10-*threo*-26,27-tetrol melted at 115 °C. When mixed with the ketone from which it was derived there was no distinct depression of m.p. ; but that it no longer contained a keto group was shown by its failure to form an oxime and by the analytical data.

The tetrahydroxy ketones, their oximes and the tetrols are presented in Table 1, together with their m.p.'s and analyses.

III. References

Bounds, D. G., Linstead, R. P., and Weedon, B. C. L. (1953).—*J. Chem. Soc.* **1953** : 2393.
Hatt, H. H., and Lamberton, J. A. (1955).—*Aust. J. Chem.* **8** : 506.
Sauer, J. C. (1951).—*Org. Synth.* **31** : 68.
Swern, D., Billen, G. N., Findley, T. W., and Scanlan, J. T. (1945).—*J. Amer. Chem. Soc.* **67** : 1786.

SHORT COMMUNICATIONS

A CRITIQUE OF THE VIEWS OF GONIKBERG AND POVKH ON THE ROLE OF SOLVATION IN A MENSCHUTKIN REACTION*

By S. D. HAMANN†

Pressure is known to have a marked effect on the rates of organic reactions in solution, and it is now apparent that many of the observed changes in rate arise from the enhanced solvation of electrically charged groups at high pressures (Buchanan and Hamann 1953 ; David and Hamann 1954 ; Burris and Laidler 1955). In principle, the pressure dependence of a reaction rate may be used to provide information about the relative extent of solvation in the initial and transition states of the reaction, but deductions of this sort must be made with great caution. To illustrate this point we shall examine the conclusions of Gonikberg and Povkh (1949) and Gonikberg (1955*a*, 1955*b*) concerning the role of solvation in the formation of *N*-ethyl pyridinium iodide from pyridine and ethyl iodide in acetone solution

$$C_5H_5N + C_2H_5I \rightarrow C_5H_5NC_2H_5^+ + I^- . \quad \ldots\ldots\ldots \quad (1)$$

Gonikberg and Povkh concluded from the high pressure kinetic measurements of Gibson, Fawcett, and Perrin (1935) that the transition state for reaction (**1**) is " unsolvated " in the sense that there is no electrostriction of the solvent in the neighbourhood of the incipient charges. This is a surprising conclusion, and one which contradicts a good deal of other evidence concerning the part played by solvents in the formation of quaternary ammonium salts (Menschutkin reactions). We shall review this evidence briefly, before considering the foundations of Gonikberg's argument. The experiments of Moelwyn-Hughes and Hinshelwood (1932) show that a Menschutkin reaction will not occur in the homogeneous gas phase ; it requires a solvent or an active surface to stabilize the transition state, and the rate of reaction is known to be roughly proportional to the dielectric constant of the solvent. Furthermore, reactions of this class have very negative entropies of activation : for example, the activation entropy for reaction (**1**) in acetone solution is -35 cal deg^{-1} mole^{-1}. This large decrease almost certainly represents the amount of entropy lost by the solvent molecules which are frozen around the partially ionic transition state (Bell 1943). The fact that the activation entropy is nearly the same as the total entropy decrease for the complete reaction shows that the transition state is almost as highly ionic and extensively solvated as the free ions (Glasstone, Laidler, and Eyring 1941).

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Turning now to an examination of Gonikberg's argument, we find that he made the reasonable assumption that the effective volume of the transition state for Menschutkin reactions is close to that of the final product. The justification for this lies in the fact that the rate of a reverse Menschutkin reaction is only slightly reduced by the pressure (Williams, Perrin, and Gibson 1936). On this basis the effective volume of activation $\Delta V^{\ddagger}$, defined by

$$\Delta V^{\ddagger} = -RT \frac{\partial \ln k}{\partial P}, \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where k is the rate constant, should be almost equal to the difference $\Delta \overline{V}$ between the partial molar volumes of the products and reactants in the same solvent. Gonikberg and Povkh (1949) measured $\Delta \overline{V}$ for reaction (**1**) in acetone at 1 atm

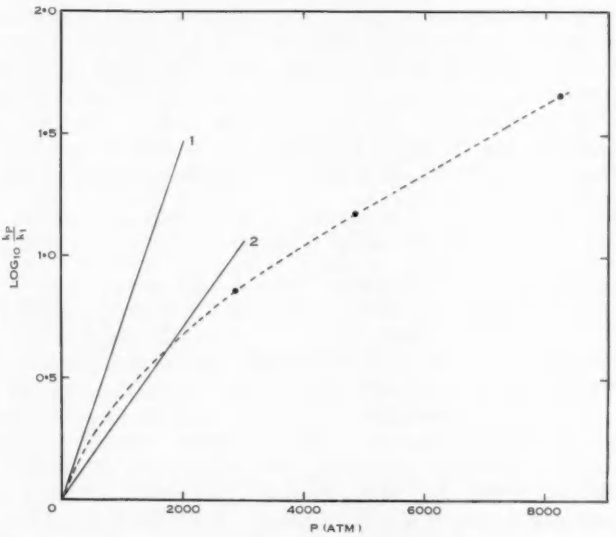

Fig. 1.—The rate of the reaction (**1**) at high pressures and at 30 °C.

and found it to be -42 cm^3 mole^{-1}, which is twice as great as the value $\Delta V^{\ddagger} = -20$ cm^3 mole^{-1} at 1 atm, estimated by Stearn and Eyring (1941) from the kinetic measurements of Gibson, Fawcett, and Perrin (1935) at 1 and 2890 atm. Confronted with this discrepancy Gonikberg and Povkh then measured the molar volume of pure liquid *N*-ethyl pyridinium iodide and found it to be greater by 26 cm^3 mole^{-1} than the partial molar volume of the same substance in acetone solution. From this they concluded that the hypothetical reaction of pyridine with ethyl iodide in acetone to give pure liquid (unsolvated) *N*-ethyl pyridinium iodide would be accompanied by almost the same volume change as the activation step for the real reaction (**1**). This is the main basis of their conclusion that the transition state is not solvated.

The flaw in this argument lies in the authors' acceptance of Stearn and Eyring's value for $\Delta V^{\ddagger}$ at 1 atm. Figure 1 shows that the slope of the curve of log *k against P* changes very rapidly at low pressures, and without measurements below 2890 atm it is quite impossible to guess the limiting slope at 1 atm, and so derive $\Delta V^{\ddagger}$ from equation (2). The line 1 corresponds to the value -42 cm^3 mole^{-1}, and the line 2 to $\Delta V^{\ddagger} = -20$ cm^3 mole^{-1}: the dotted curve is one which was drawn arbitrarily by Williams, Perrin, and Gibson (1936). It is clear that Stearn and Eyring's choice of -20 cm^3 mole^{-1} is no better, and probably worse than the value -42 cm^3 mole^{-1} which is Gonikberg and Povkh's measured volume change for the formation of the solvated salt.

The difficulty of estimating volume changes at 1 atm from rate and equilibrium measurements at high pressures is emphasized by the following values for the difference between the partial molar volumes of piperidine in the ionized and un-ionized forms in methanol at 25 °C (Hamann and Strauss 1956):

P (atm)	1	50	250	750	1500	2500
$\Delta\bar{V}$ (cm^3 mole^{-1}) ..	$-49{\cdot}5$*	-46†	-32†	-28†	-24†	-19†

Obviously, any attempt to estimate $\Delta\bar{V}$ at 1 atm from measurements above 2500 atm would be doomed.

References

Bell, R. P. (1943).—*J. Chem. Soc.* **1943**: 629.

Buchanan, J., and Hamann, S. D. (1953).—*Trans. Faraday Soc.* **49**: 1425.

Burris, C. T., and Laidler, K. J. (1955).—*Trans. Faraday Soc.* **51**: 1497.

David, H. G., and Hamann, S. D. (1954).—*Trans. Faraday Soc.* **50**: 1188.

Gibson, R. O., Fawcett, E. W., and Perrin, M. W. (1935).—*Proc. Roy. Soc.* A **150**: 223.

Glasstone, S., Laidler, K. J., and Eyring, H. (1941).—"The Theory of Rate Processes." p. 418. (McGraw-Hill Book Co. Inc.: New York.)

Gonikberg, M. G. (1955*a*).—*Adv. Chem. Moscow* **24**: 14.

Gonikberg, M. G. (1955*b*).—*C.R. Acad. Sci. U.R.S.S.* **102**: 117.

Gonikberg, M. G., and Povkh, G. S. (1949).—*J. Phys. Chem. Moscow* **23**: 383.

Hamann, S. D., and Lim, S. C. (1954).—*Aust. J. Chem.* **7**: 329.

Hamann, S. D., and Strauss, W. (1956).—*Disc. Faraday Soc.* (in press).

Moelwyn-Hughes, E. A., and Hinshelwood, C. N. (1932).—*J. Chem. Soc.* **1932**: 230.

Stearn, E. A., and Eyring, H. (1941).—*Chem. Rev.* **29**: 504.

Williams, E. G, Perrin, M. W., and Gibson, R. O. (1936).—*Proc. Roy. Soc.* A **154**: 684.

* From density measurements at 1 atm (Hamann and Lim 1954).

† From the relation $\Delta\bar{V} = -RT\,\dfrac{\partial \ln K}{\partial P}$, where K is the molal ionization constant.

COMPRESSIONS OF ORGANIC LIQUIDS AND THEIR MIXTURES WITH WATER*

By J. E. Stutchbury†

Although a large number of measurements of the compressions‡ of pure organic substances have been made little is known about the compressions of their mixtures with water. Moesveld (1923) and Gibson (1935) measured the compressions of aqueous solutions of ethyl alcohol and methyl alcohol respectively, and Richards and Chadwell (1925) measured the compressions of aqueous solutions of ether and methyl acetate. More recently, Newitt and Weale (1951) measured the compressions of the mixtures water–*n*-propyl alcohol and water–acetone. All these workers discussed the fact that the addition of a small amount of an organic liquid to water, decreases the compression until a minimum value is reached. The inverse effect would be expected, since organic liquids are more compressible than water. This phenomenon of the decrease in the compression of water was discovered by Drucker (1905) in measurements on the compressions of aqueous solutions of acetic acid and the three chloroacetic acids. No information on the compressions of aqueous solutions of *iso*propyl alcohol and *tert.*-butyl alcohol has been recorded in the literature, so the present compressions have been measured to complete the homologous series of methyl-substituted carbinols. Measurements of the compression of aqueous solutions of ethyl alcohol, acetone, and pyridine have also been made; new values are given in the first two instances, while pyridine has not previously been investigated.

For pure methyl alcohol Gibson (1935) has published compression results which are high in comparison with those of Amagat (1893), Richards *et al.* (1912), and Bridgman (1913–14*a*). Because of these discrepancies in the literature, new values of the compression of methyl alcohol have been determined in the present work.

Experimental

(*a*) *Materials.*—The liquids used were carefully purified and fractionated through a 1 in. column, 2 ft long, packed with glass helices, fractions boiling within a range of not more than 0·15 °C being collected. The physical constants obtained for the liquids are listed below, and are compared with the accepted values of the Carnegie Institute of Technology, Pittsburgh (1954), except for pyridine, where comparison is made with Timmermans's (1950) data. Boiling

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‡ The compression is defined as $\Delta V/V$, where ΔV is the contraction produced by a pressure P/atm, and V is the volume at 1 atm.

points are corrected. The refractive indices were measured by an immersion refractometer (Table 1).

All samples were stored in tightly stoppered bottles, and used immediately after distillation. Conductivity water was used in preparation of all solutions.

(*b*) *Apparatus.*—An Aimé piezometer similar to those described by Bridgman (1949) was used for compression measurements. The steel bomb, in which the piezometer was enclosed, was maintained at a constant temperature of 30 °C in an oil-bath. The pressure was read directly on calibrated Bourdon gauges.

(*c*) *Method.*—All measurements were made in a single piezometer, the volume of which was regularly calibrated by weighing the piezometer filled with water.

TABLE 1

COMPARISON OF BOILING POINTS AND REFRACTIVE INDICES

Liquid	Boiling Point (°C)	Boiling Point (lit.) (°C)	n_D^{25}	n_D^{25} (lit.)
Methyl alcohol	—	—	1·32654	1·32657
Ethyl alcohol	78·35	78·33	1·35949	1·35941
*iso*Propyl alcohol	82·30	82·35	1·37500	1·37492
tert.-Butyl alcohol	82·25	82·57	1·38507	1·3851
Acetone	56·18	56·33	1·35632	1·35628
Pyridine	115·65	115·58	1·50716	1·5072

The bomb was let stand in the oil-bath for 30 min to reach the temperature of 30 °C which was regulated within $\pm 0{\cdot}2$ °C. When applying pressure it was found necessary to maintain a steady, continuous increase, which was controlled by throttling one valve in the system, leaving a reservoir of pressure on the side of the valve remote from the bomb. This operation was completed over a period of 20 min to allow time for the heat of compression to dissipate. Shorter compression periods than this resulted in low apparent compressions.

Immediately the required pressure was reached, it was released. Excess mercury in the cap of the piezometer was quickly removed, for delays at this point could cause the piezometer to cool slightly and suck in mercury. The mercury inside the piezometer was washed with absolute alcohol, dried *in vacuo*, and then weighed. Occasionally a fine white film, of unknown composition, was found on the surface of the mercury, but as this could readily be washed away with water, it was disregarded.

(*d*) *Calculations.*—These follow the pattern of work described by Bridgman (1911–12). The volume of 1 g of mercury is recorded (Smithsonian Tables 1920) as being 0·073957 ml at 30 °C. The relative change in the volume of mercury at high pressure was found by graphical interpolation, based on the results of Bett, Weale, and Newitt (1954). The change in the volume of the piezometer was found by using the value for the compression of 18/8 stainless steel given by Keyes (1932–33).

Results

Compressions of aqueous solutions of *iso*propyl alcohol and pure methyl alcohol have been measured at 30 °C with pressures up to 2000 atm, and these results are listed in Tables 2 and 3.

TABLE 2

COMPRESSIONS OF AQUEOUS SOLUTIONS OF *iso*PROPYL ALCOHOL AT 30 °C

$\Delta V/V \times 10^3$

*iso*Propyl Alcohol (mole %)	Pressure (atm)				
	250	500	1000	1500	2000
0·00	12·1	21·9	41·0	55·4	68·2
		23·9	42·6		
			41·6		
			41·8		
5·35		20·1	37·4	49·0	61·8
13·05		19·6	37·9	54·7	67·9
		20·3			67·1
33·29		27·3	50·4	66·1	83·1
66·24		34·7	60·9	81·4	97·1
88·50		40·4	69·0	88·7	107·8
					107·3
100·00	24·8	41·5	69·9	90·8	107·7
	23·0	42·1	70·7		112·5
		41·2			

Compressions of aqueous solutions of ethyl alcohol, *teri.*-butyl alcohol, acetone, and pyridine were measured at 30 °C, at 1000 atm and these results, together with those for the compressions of aqueous solutions of *iso*propyl alcohol, are given in Table 4. The results in Table 4 have been plotted in Figure 1.

TABLE 3

COMPRESSION OF METHYL ALCOHOL AT 30 °C

$\Delta V/V \times 10^3$

Pressure (atm)	100	200	300	500	800	1000	1500	2000
Compression ..	11·0	20·1	28·3	44·1	67·5	78·8	103·5	123·6

Discussion

(*a*) *Errors.*—The variation in the temperature of the oil-bath was not more than 0·2 °C, which was negligible, but the temperature rise in the piezometer during compression may well have been 1–2 °C. The Bourdon pressure gauges had an accuracy better than 0·25 per cent. The capillary of the piezometer was extremely small, so that the volume of the last drop of mercury which clung there, and which was expelled on release of pressure, could be discounted. In

all, the greatest errors were due to (i) uneven compression which led to high results, and (ii) insufficient time for the heat of compression inside the piezometer to dissipate which gave low results.

TABLE 4

COMPRESSIONS OF AQUEOUS SOLUTIONS OF ORGANIC LIQUIDS AT 1000 ATM AND 30 °C

Ethyl Alcohol		*iso*Propyl Alcohol		*tert.*-Butyl Alcohol		Acetone		Pyridine	
Mole %	$\Delta V/V \times 10^3$	Mole %	$\Delta V/V \times 10^3$	Mole %	$\Delta V/V \times 10^3$	Mole %	$\Delta V/V \times 10^3$	Mole %	$\Delta V/V \times 10^3$
0·00	41·8	0·00	41·8	0·00	41·8	0·00	41·8	0·00	41·8
1·81	38·5	5·35	37·4	1·53	36·3	5·73	37·8	4·20	37·2
4·13	36·7	13·05	37·9	3·00	34·7	13·50	38·1	9·75	36·6
12·20	37·5	33·29	50·4		35·4	23·10	41·8	18·42	37·5
22·60	39·3	63·24	60·9	6·26	36·9	41·56	52·8	29·72	38·9
40·06	48·1	88·50	69·0	11·64	41·5	58·35	63·0	52·77	41·9
63·08	57·3	100·00	70·3	16·03	42·9	76·89	71·6	72·56	44·9
82·82	65·1			27·89	49·8	100·00	79·4	100·00	51·8
100·00	70·7			50·18	59·7				
				100·00	Freezes				

The reproducibility of the results was variable. For example, the four measurements made on water at 1000 atm. agree within 4 per cent., while the three results for *iso*propyl alcohol at 500 atm agree within 2 per cent. Errors in the compressions measured at 250 atm were as great as 10 per cent. and for

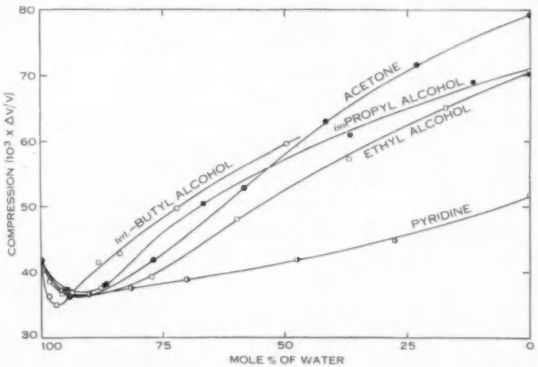

Fig. 1.—Compressions of various mixtures at 1000 atm and 30 °C.

this reason measurements at this pressure were discontinued. The variation of the error could possibly be explained by the inclusion of air bubbles in the piezometer, which even the most careful filling may have failed to remove. This effect was probably most important for water, which failed to wet completely the steel surface of the piezometer.

(*b*) *Comparison with Other Work.*—The results of the present work are in close agreement with those measurements of earlier workers. Gibson's (1935) data for methyl alcohol appear to be incorrect, whilst the present results are in agreement with those of Bridgman (1913–14*a*) and Amagat (1893).

Conclusion

It is apparent from Figure 1 that small concentrations of alcohols, acetone, and pyridine all reduce the compression of water, although the solutes are more compressible than water. The origin of the effect probably lies in the complex structure of water, and no satisfactory explanation can be offered at this stage.

The author wishes to thank Dr. S. D. Hamann for his guidance and helpful suggestions in this work, as well as in the preparation of this paper. He is indebted to Mr. H. G. David for designing the piezometer and assembling the high pressure apparatus.

References

Amagat, E. H. (1893).—*Ann. Chim. phys.* **29**: 508.
Bett, K. E., Weale, K. E., and Newitt, D. M. (1954).—*Brit. J. Appl. Phys.* **5**: 243.
Bridgman, P. W. (1911–12).—*Proc. Amer. Acad. Arts Sci.* **47**: 357.
Bridgman, P. W. (1912–13).—*Proc. Amer. Acad. Arts Sci.* **48**: 338.
Bridgman, P. W. (1913–14*a*).—*Proc. Amer. Acad. Arts Sci.* **49**: 40.
Bridgman, P. W. (1913–14*b*).—*Proc. Amer. Acad. Arts Sci.* **49**: 43.
Bridgman, P. W. (1913–14*c*).—*Proc. Amer. Acad. Arts Sci.* **49**: 60.
Bridgman, P. W. (1930–31).—*Proc. Amer. Acad. Arts Sci.* **66**: 215.
Bridgman, P. W. (1949).—" Physics of High Pressure." p. 101. (Bell & Sons: London.)
Carnegie Inst. of Technology, Pittsburgh (1954).—" Selected Values of Properties of Hydrocarbons and Related Compounds." (Carnegie Press: Pittsburgh.)
Drucker, K. (1905).—*Z. phys. Chem.* **52**: 661.
Gibson, R. E. (1935).—*J. Amer. Chem. Soc.* **57**: 1551.
Keyes, F. G. (1932–33).—*Proc. Amer. Acad. Arts Sci.* **68**: 505.
Moesveld, A. L. Th. (1923).—*Z. phys. Chem.* **105**: 453.
Newitt, D. M., and Weale, K. E. (1951).—*J. Chem. Soc.* **1951**: 3092.
Richards, T. W., and Chadwell, H. M. (1925).—*J. Amer. Chem. Soc.* **47**: 2996.
Richards, T. W., Stull, W. N., Mathews, J. H., and Speyers, C. L. (1912).—*J. Amer. Chem. Soc.* **34**: 988.
Smithsonian Tables (1920).—*Smithsonian Misc. Coll.* **71**: 121.
Timmermans, J. (1950).—" Physicochemical Constants of Pure Organic Compounds." (Elsevier Publ. Co.: New York.)

VAPOUR PHASE CHROMATOGRAPHIC SEPARATION OF AROMATICS FROM SATURATED AND OLEFINIC HYDROCARBONS*

By J. R. Anderson† and K. H. Napier†

Gas-liquid partition chromatography (cf. James and Martin 1952; Ray 1954) has proved to be an attractive technique for the analysis of hydrocarbon mixtures. The use of a non-polar stationary phase such as liquid paraffin or "Apiezon" oil results in the elution of the components in the order of their boiling points and this may cause difficulty in separation when the differences in volatility are small. Thus *cyclo*hexane (b.p. 81·4 °C/760 mm) and benzene (b.p. 80·1 °C/760 mm) were only partly resolved at 100 °C on a column 4 ft long with "Apiezon" oil as the stationary phase. It has been found that the polyether glycols or their derivatives provide a liquid stationary phase which is selective for the separation of aromatics from saturated and olefinic hydrocarbons of comparable boiling points due to the greater interaction of the aromatic with the polyether.

The equipment used was essentially similar to that described by Ray (1954), except the signal from the thermal conductivity cell which was energized from a D.C. source was amplified by a fedback galvanometer amplifier and recorded on a 1 mA Evershed and Vignols recorder. Later the signal was fed directly to a 5 mV Speedomax recorder. The thermal conductivity cell was operated at room temperature and pressure. The liquid stationary phase (40% w/w) was supported on 44–60 mesh alumina which was obtained by grinding and sieving 16–32 mesh "Type A" alumina from Peter Spence and Sons Ltd., Widnes, England. This support was found to impede the gas flow much less than the keiselguhr packings of James and Martin (1952) and Ray (1954). When carrying the liquid polyether, the alumina was quite inert and formed a highly convenient supporting material. Nitrogen, dried over silica gel, was used as the carrier gas.

Figure 1 (*a*) shows the chromatogram obtained from a mixture of *cyclo*hexane, benzene, and *cyclo*hexane (b.p. 83 °C/760 mm) with a stationary phase of triethylene glycol (T.G.) at 100 °C, while Figure 1 (*b*) shows the same mixture over polythylene glycol cresyl ether (P.G.C.E.) at 131 °C. Benzene, despite its lower boiling point, appears last in both cases and is easily separated from the other two. The presence of one double bond has comparatively little influence on the behaviour of *cyclo*hexene compared to *cyclo*hexane although these are separated rather better on P.G.C.E. than on T.G. The similar behaviour of the two stationary phases with benzene shows that it is the ether link and not the hydroxyl group which is primarily responsible for interaction

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with the aromatic. A comparison of Figures 1 (*c*) and 1 (*d*) shows that for equal flow rates the separation of benzene from *cyclo*hexane is better at 100 °C than 131 °C.

On P.G.C.E. at 132 °C, *cyclo*hexane is not separated from methyl*cyclo*hexane (b.p. 100·3 °C/760 mm) although benzene is easily separated from toluene

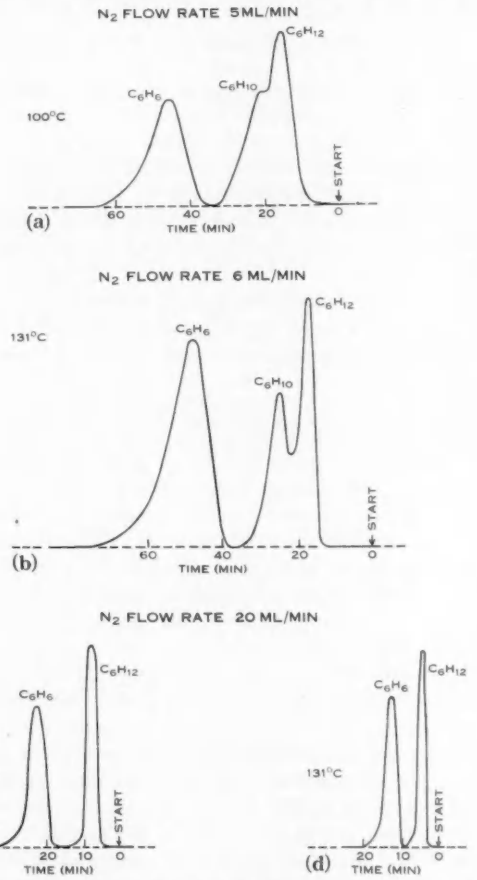

Fig. 1.—Chromatograms on (*a*) triethylene glycol; (*b*), (*c*), (*d*) polyethylene glycol cresyl ether.

(b.p. 110·8 °C/760 mm). The chromatogram from a four component mixture containing benzene, toluene, *cyclo*hexane, and methyl*cyclo*hexane is shown in Figure 2. That a saturated hydrocarbon of b.p. 100 °C appears in the eluate well before benzene suggests that this stationary phase may solve the problem of the analysis of the products from a catalytic reforming process for petroleum

(Keulemans, Kwantes, and Zaal 1956), where on a number of stationary phases of various types interference between these components was found.

The enhanced interaction of the polyether with an aromatic molecule is probably due to dipole-induced dipole forces involving the comparatively

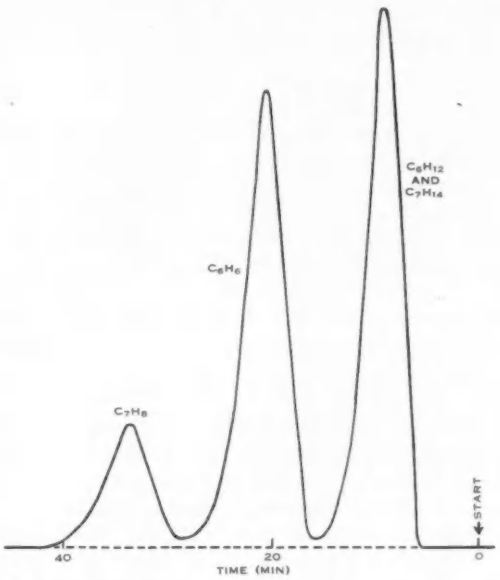

Fig. 2.—Chromatogram on polyethylene glycol cresyl ether.

easily polarizable π-electrons of the benzene molecule. A contribution from charge-transfer forces (Mulliken 1952*a*) is not likely to be significant since ethers and benzene are both type *n* donor molecules on the classification of Mulliken (1952*b*).

References

James, A. T., and Martin, A. J. P. (1952).—*Biochem. J.* **50**: 679.
Keulemans, A. I. M., Kwantes, A., and Zaal, P. (1956).—*Anal. Chim. Acta* **13**: 357.
Mulliken, R. S. (1952*a*).—*J. Amer. Chem. Soc.* **74**: 811.
Mulliken, R. S. (1952*b*).—*J. Phys. Chem.* **56**: 801.
Ray, N. H. (1954).—*J. Appl. Chem.* **4**: 21.

THE ANTHOCYANINS OF THE LEAVES AND YOUNG BARK OF *EUCALYPTUS SIEBERIANA* F. MUELL.*

By W. E. Hillis†

A chromatographic examination was made (Hillis 1956) of the leaves and young bark of *Eucalyptus capitellata* Sm. (brown stringybark), *E. elaeophora* F. Muell. (long-leaved box), *E. maculata* Hook. (spotted gum), *E. obliqua* L'Herit. (messmate), *E. regnans* F. Muell. (mountain ash), and *E. sieberiana* F. Muell. (silvertop ash). It revealed that the two main anthocyanins in the leaves were, in all cases, different from the main anthocyanin in the outer layer of young bark.

The anthocyanins have now been extracted from samples of *E. sieberiana*. Owing to the instability of the concentrates and the persistence of impurities with similar R_F values, attempts to isolate the pure anthocyanins were unsuccessful. However, after separation on a cellulose column and purification with solvents the components were in a sufficiently pure form to permit their identification by chromatographic comparison, colour, and other tests.

The main anthocyanin in the bark has been identified as chrysanthemin (cyanidin-3-glucoside) and the other anthocyanin appeared to be delphinidin-3-glucoside, but sufficient amounts of it could not be obtained for detailed examination.

The two main anthocyanins in the leaves have been identified as delphinidin-3-glucoside and delphin (delphinidin-3,5-diglucoside). During the isolation of these pigments a concentrate of a third pigment was collected. Under ultraviolet light it possessed a strong pelargonidin-like salmon-pink fluorescence.

Experimental

(*a*) *Isolation of Anthocyanins.*—The fresh red leaves or the outer layers of fresh red bark from young stems of *E. sieberiana* were shaken with successive lots of methanol until all the extractives were removed. The extracts were evaporated under reduced pressure at 60 °C, the residue washed with methanolic-hydrochloric acid (5%), and the insoluble material removed. The liquor was chromatographed on columns of cellulose pulp (" Solka Floc " (200 mesh) ; Brown Co., Boston, U.S.A.) after it had been purified by the method of Campbell, Work, and Mellanby (1951). The column was developed with the organic layer of ethyl acetate : acetic acid : formic acid : water (18 : 3 : 1 : 4), sucked dry, extruded, the red bands separated, and extracted with methanol : 1% HCl (1 : 1). The anthocyanins were further purified by rechromatographing the concentrated extracts on a smaller column.

Ether was added to the methanolic extract and the precipitated anthocyanin removed from the accompanying insoluble colourless material by washing several times with a small quantity of methanol. This process was repeated several times.

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TABLE 1

R_F VALUES OF THE ANTHOCYANINS AND CORRESPONDING ANTHOCYANIDINS

Substance	BuOH : AcOH : H_2O (4 : 1 : 5)*	BuOH : 2N HCl*	*m*-Cresol : AcOH : H_2O (50 : 2 : 48)*	EtOAc : 80% HCOOH : H_2O (8 : 2 : 3)	PrOH : 2N HCl (1 : 1)	HCl : HOAc : H_2O (3 : 30 : 10)†
Leaf anthocyanin I	0·09	0·07	0·05	0·10	0·29	—
Partly hydrolysed leaf anthocyanin I	0·16	0·16	—	—	—	—
Leaf anthocyanin II	0·16	0·16	0·13	0·28	—	—
Anthocyanidin from I and II ..	0·35	0·38	—	—	—	0·32
Leaf anthocyanin III	0·16	0·10	0·46	0·28	0·30	—
Anthocyanidin from III ..	—	0·56	—	—	—	0·54
Bark anthocyanin..	0·31	0·21	0·22	0·26	0·33	0·56
Bark anthocyanidin	0·57	0·65	—	0·73	—	0·56
Chrysanthemin	0·32	0·21	0·22	0·28	0·34	0·56
Cyanidin	0·57	0·65	—	0·75	—	0·56
Delphin	0·07	0·09	0·04	0·12	0·29	—
Delphinidin-3-glucoside	0·14	0·16	0·13	0·28	—	—
Delphinidin	0·35	0·35	—	—	—	0·32

* Bate-Smith (1951).

† Bate-Smith (1954).

When dilute ethanolic HCl solutions of leaf anthocyanins I and II were slowly evaporated, crystals with a deep blue metallic lustre separated. When chromatographic sheets were stored for a few days these two anthocyanins became blue in contrast to the other anthocyanins which remained red.

(*b*) *Identification of Anthocyanins.*—Ethanolic solutions of the anthocyanins were hydrolysed for 10 min in 20% HCl at 100 °C in a sealed tube. The aglycones were extracted with amyl alcohol, and examined by distribution tests (Robinson and Robinson 1931, 1932) and by chromatographic behaviour in several solvents (Table 1). The known pigments run for comparison at the same time were chrysanthemin from the red autumnal foliage of *Acer* sp. and the flowers of the red carnation (*Dianthus* sp.), and delphin from *Delphinium* sp. They were hydrolysed to obtain the corresponding anthocyanidin.

The aqueous solutions after hydrolysing the anthocyanins were neutralized with Ag_2CO_3, centrifuged, and concentrated *in vacuo*. The concentrates were examined by paper chromatography using the solvent systems of butanol : acetic acid : water (4 : 1 : 5), ethyl acetate : 80% formic acid : water (8 : 2 : 3), and phenol : water. The sugars were detected with *p*-anicidine hydrochloride (Hough, Jones, and Wadman 1950) and glucose was found to be the only sugar liberated on acid hydrolysis of the anthocyanins.

A concentrate of the deep blue needles of leaf anthocyanin I (R_F 0·09 in butanol : acetic acid : water) was partly hydrolysed for 30 min with $2N.H_2SO_4$ at 100 °C. The principal product was identical with the leaf anthocyanin II R_F 0·16; also present were glucose and another component with properties similar to delphinidin. Authentic delphinidin-3,5-diglucoside behaved identically. The minor anthocyanin in the bark and leaf anthocyanin II showed identical properties on chromatographic examination.

Whilst purifying leaf anthocyanin II, another component, III, became evident; the R_F values of these substances in butanol : acetic acid : water were almost identical. Under ultraviolet light, component III possessed a characteristic salmon-pink fluorescence very similar to that of pelargonidin. All attempts to purify III failed, but an examination of a fraction containing an appreciable amount of it showed that glucose was the only sugar present. Lack of material prevented further study.

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References.

Bate-Smith, E. C. (1951).—Biochem. Soc. Symp. No. 3, p. 62.
Bate-Smith, E. C. (1954).—*Biochem. J.* **58**: 122.
Campbell, P. N., Work, T. S., and Mellanby, E. (1951).—*Biochem. J.* **48**: 109.
Hillis, W. E. (1956).—*Aust. J. Biol. Sci.* **9**: 263.
Hough, L., Jones, J. K. N., and Wadman, W. H. (1950).—*J. Chem. Soc.* **1950**: 1702.
Robinson, G. M., and Robinson, R. (1931).—*Biochem. J.* **25**: 1687.
Robinson, G. M., and Robinson, R. (1932).—*Biochem. J.* **26**: 1647.

DYSOXYLONENE*

By R. O. Hellyer† and H. H. G. McKern†

The volatile wood-oil of *Dysoxylon fraseranum* Benth. (" rosewood ") was first examined by Penfold (1927) who found it to consist predominantly of sesquiterpenes. Two oils distilled from trees from the Chillingham district, N.S.W., were found to contain a large proportion of a fraction yielding crystalline dihydrochlorides, m.p. 118–119 °C, $[\alpha]_D^{20}$ +41·1 and −43·65°. However, an oil from the Comboyne district was characterized by giving an optically-inactive dihydrochloride, m.p. 108–109 °C. The optically-inactive cadinene-type sesquiterpene regenerated from this derivative had b_{10} 136–137 °C; d_{15}^{15} 0·9236; n_D^{20} 1·5063, and was named " dysoxylonene ".

We have examined a freshly-distilled oil of this species from Comboyne and also obtained an optically-inactive white crystalline dihydrochloride, but of m.p. 105–106 °C (Found: C, 64·7; H, 9·5; Cl, 25·8%. Calc. for $C_{15}H_{26}Cl_2$: C, 65·0; H, 9·4; Cl, 25·6%). Digestion with glacial acetic acid and sodium acetate on the steam-bath resulted in the formation of an optically-inactive sesquiterpene, b_{10} 135–136 °C; d_{15}^{15} 0·9247; n_D^{20} 1·5069. The infra-red spectrum of the dysoxylonene dihydrochloride was found to be identical with that of the dihydrochloride, m.p. 119 °C, prepared from a (+)-cadinene from the volatile leaf-oil of *Eucalyptus maculata* Hook.

Kafuku, Ikeda, and Hata (1935) isolated from *Lantana camara* L. a cadinene-like sesquiterpene, micranene, yielding a dihydrochloride, m.p. 105·5–106·5 °C, and which was shown by Sebe (1940) to be (±)-cadinene. Hence, both micranene and dysoxylonene are optically-inactive cadinenes, and the use of these trivial names should be discontinued.

References

Kafuku, K., Ikeda, T., and Hata, C. (1935).—*J. Chem. Soc. Japan* **56**: 1184.
Penfold, A. R. (1927).—*J. Proc. Roy. Soc. N.S.W.* **61**: 337.
Sebe, Y. (1940).—*J. Chem. Soc. Japan* **61**: 1269.

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INDEX